Correlation of Imaging features of Infiltrating Breast Carcinoma on Mammography and Ultrasound with the molecular subtypes (Luminal A, Luminal B, HER2neu enriched and TNBC)

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ALL INDIA INSTITUTE OF MEDICAL SCIENCES

BHOPAL

For the award of the degree of **Doctor of Medicine (MD)** in the subject of

RADIODIAGNOSIS

by

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(October 2024)

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INTRODUCTION

Breast cancer is now the first most common cause of cancer-related mortality, with around 2.3 million new cases worldwide, based on GLOBOCAN 2022 data (1). Globally, women lose more disability-adjusted life years (DALYs) to breast cancer than to any other form of cancer. It affects women in every country and can occur at any age after puberty, though the risk increases with age.

Probably, the most important factor in reducing mortality associated with breast cancer is an early diagnosis of cancer. Triple Assessment in breast cancer is designed for rapid diagnosis of symptomatic patients via clinical, radiological and histopathological examination (2). Imaging is an important part of breast cancer clinical protocols and can provide a variety of information about morphology, structure, metabolism and functions. Mammography is the only widely used imaging modality for breast cancer screening. Several large randomized clinical trials have shown that mammography reduces mortality from breast cancer. The role of B-mode ultrasound in breast imaging has been largely limited to applications such as distinguishing between cystic versus solid masses, evaluation of palpable masses, and for needle core biopsy of masses. However, in recent years, the number of indications has been greatly expanded- including the imaging of dense breast for screening as well as diagnosis, and breast ultrasonography is now an essential modality in breast imaging.

Several ultrasounds and mammographic characteristics of malignant breast lesions, such as hypo-echogenicity, irregular shape, vertical orientation, presence of spicules, posterior acoustic shadowing, association with calcifications, and increased vascularity, can be associated with various breast cancer subtypes.

The BI-RADS (Breast Imaging-Reporting and Data System) is a risk evaluation tool created by the American College of Radiology. It offers a well-established vocabulary and reporting framework for breast imaging, applicable to mammography, ultrasound, and MRI. BI-RADS standardizes the terminology used to describe findings in breast imaging.

Histological grading of Infiltrating Ductal Carcinoma is done with Nottingham modification of the Bloom-Richardson system, which is based on

(a) tumor tubule formation,

(b) number of mitotic figures in most active areas and

(c) nuclear pleomorphism (3).

Molecular classification independently from histological subtypes, invasive breast cancer is divided into Luminal A, Luminal B, HER2Neu enriched and TNBC.
Immunohistochemistry plays an important role in detecting biomarker expression concerning breast pathology and it has demonstrated a strong correlation between receptor expression and patient's response to therapy. Gene profile analysis is important but expensive and difficult to perform routinely. ER, PR and HER2 and Ki67 are the most common immunomarkers used in determining the prognosis of BC and for deciding the therapeutic strategy. Imaging features of different molecular subtypes have been shown to be different with different molecular receptors.
However, obtaining immunohistochemistry is time-consuming and also may not be available in various parts of our country. This motivated us to explore whether on radiological evaluation of breast lesions, information about the molecular subtypes can be derieved.

REVIEW OF LITERATURE

Milk secreting glands, present in both females and males in humans, are for nourishing offspring. The mammary glands are, however are functional only in the postpartum female. For understanding the radiological appearance and associated pathologies, understanding of the anatomy of the breast is important.

ANATOMY OF THE BREAST:

The breast is situated in the superficial fascia of the pectoral region. Vertically, it extends from the second to the sixth rib. Horizontally, it extends from the lateral border of the sternum to the midaxillary line.

The breast is anatomically divided into four quadrants, i.e. upper medial, upper lateral, lower medial and lower lateral. A small extension of the upper lateral quadrant called the axillary tail of Spence, passes through an opening in the deep fascia and lies in the axilla. (Fig. 1). The opening is called foramen of Langer. The breast lies on the deep fascia (pectoral fascia) covering the pectoralis major. The breast is separated from the pectoral fascia by loose areolar tissue, called the retro-mammary space. Because of the presence of this loose tissue, the normal breast can be moved freely over the pectoralis major. (Fig. 2). (5)

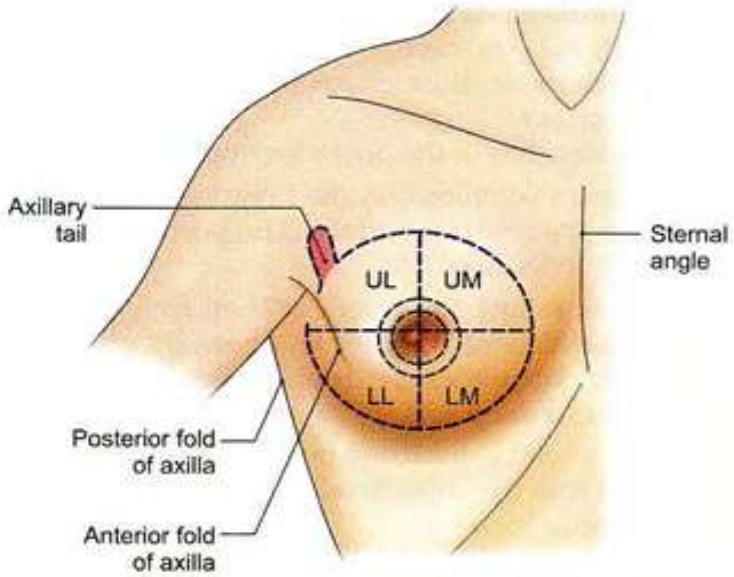


Figure 1: Image from Anatomy for Students- By Dr. B.D. Chaurasiya

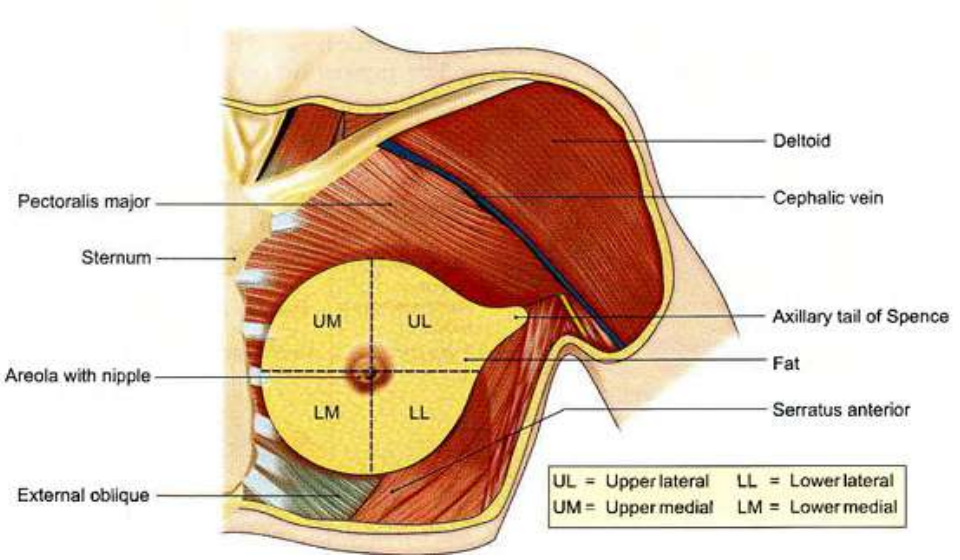


Figure 2 : Image from Anatomy for Students- By Dr. B.D. Chaurasiya

The nipple is a conical projection present just below the centre of the breast at the level of the fourth intercostal space about 10 cm from the midline. The nipple gives way to 15 to 20 lactiferous ducts. The skin surrounding the base of the nipple is pigmented and forms a circular area called the areola. Below the areola lie lactiferous sinus where stored milk is seen.

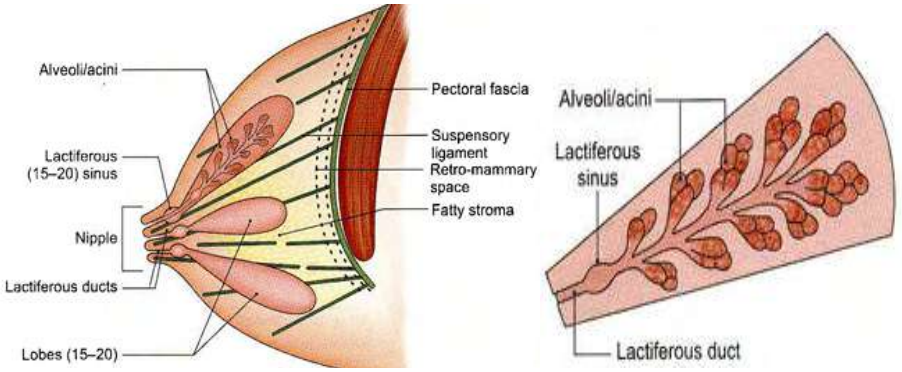


Figure 3a, 3b

Each breast contains 15–20 lobes each consist of many lobules. Each lobe has a cluster of alveoli and is drained by a single lactiferous duct. The lactiferous ducts converge towards and end on the nipple. (Figure 3a, 3b)

Near its termination each duct has a dilatation called lactiferous sinus. Myo-epitheliocytes facilitate the passage of milk from the alveoli towards the nipple. Supporting stroma is fibro-fatty. The fibrous septae are known as the suspensory

ligaments of Cooper, which anchor the gland to the pectoral fascia (Fig. 3a). Lymphatic drainage of breast is very important. Lymph from the breast drains into the following lymph nodes (Fig.4a) - the axillary lymph nodes, chiefly the anterior (or pectoral) group, the posterior, lateral, central and the internal mammary (parasternal) nodes. The superficial lymphatics drain the skin over the breast except for the nipple and areola. The lymphatics pass radially to the surrounding lymph nodes. The deep lymphatics drain the parenchyma of the breast. They also drain the nipple and areola. A plexus of lymph vessels is present deep to the areola. This is the subareolar plexus of Sappey (Fig. 4b).

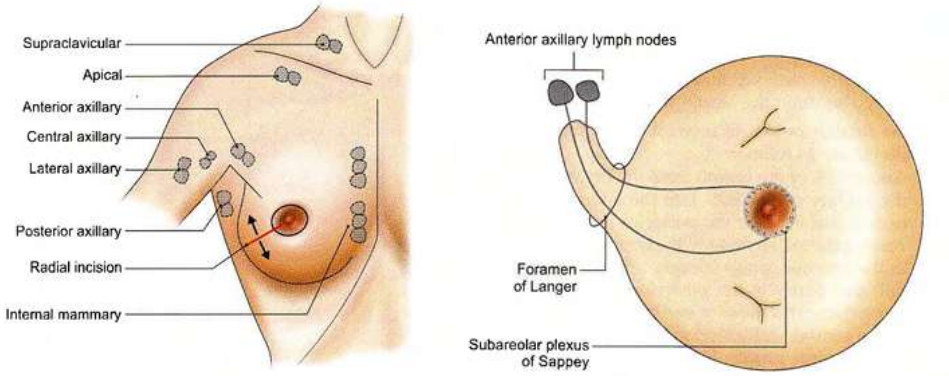


Figure 4a, 4b

The subareolar plexus and most of the lymph from the breast drains into the anterior or pectoral group of lymph nodes. The lymphatics from the deep surface of the breast pass through the pectoralis major muscle and the clavipectoral fascia to reach the apical nodes, and also to the internal mammary nodes. Lymphatics from the lower and inner quadrants of the breast may communicate with the subdiaphragmatic and sub-peritoneal lymph plexuses after crossing the costal margin and then piercing the anterior abdominal wall through the upper part of the linea alba.

EPIDEMIOLOGY OF BREAST CANCER:

Breast cancer is one of the most prevalently diagnosed cancers. Female sex is associated with a heightened risk of breast cancer primarily because of enhanced hormonal stimulation. Unlike men, who present insignificant estrogen levels, women have breast cells that are very vulnerable to hormones (estrogen and progesterone in particular) and any disruptions in their balance. Circulating estrogens and androgens are positively correlated with an increased propensity of developing breast cancer. Currently, about

80% of patients getting breast cancer are women aged more than fifty years of age, while at the same time, more than 40% are those more than 65 years old. The risk of developing breast cancer escalates as follows — 1.5% risk at age forty, 3% at age fifty, and more than 4% at age seventy (5). Interestingly, a relationship between a particular molecular subtype of cancer and a patient's age was observed –aggressive resistant triple-negative breast cancer subtype is most commonly diagnosed in groups under 40 age, while in patients >70, it is luminal A subtype.(4)

RISK FACTORS ASSOCIATED WITH CARCINOMA BREAST

A positive family history of carcinoma breast constitutes a significant factor associated with an increased risk of breast cancer. About 13–19% of breast cancer patients have a first-degree relative affected by breast cancer (5).

Numerous studies confirmed a strict relationship between exposure to endogenous hormones—estrogen and progesterone in particular—and excessive risk of breast cancer in females. Therefore, the events such as pregnancy, breastfeeding, first menstruation, and menopause, along with their duration and the concomitant hormonal imbalance, are important as a potential inducer of carcinogenic events in the breast microenvironment. The first full-term pregnancy at an early age (especially in the early twenties), along with a subsequently increasing number of births, is associated with a reduced risk of breast cancer (6).

Besides, an early age of first menstruation could result in a poorer prognosis. Contrarily, early menopause, whether natural or surgical, lowers the breast cancer risk.

Generally, greater breast tissue density correlates with greater breast cancer risk; this trend is observed both in premenopausal and postmenopausal females. Greater density of breasts is observed in females of younger age and lower BMI who are pregnant or during the breastfeeding period, as well as during the intake of hormonal replacement therapy. (7) In a study conducted by **Park et al. (2014) in the** Korean women population, the risk of breast cancer increased progressively with the increment of volumetric density grade (VDG) in postmenopausal women ($p < 0.001$).(6)

A personal history of breast cancer is associated with a greater risk of renewed cancerous lesions within the breasts, such as atypical hyperplasia, carcinoma in situ, or many other proliferative or non-proliferative lesions, also increasing the risk significantly (8), (9). The histologic classification of benign lesions and a positive family history of breast cancer are two factors that greatly increase breast cancer risk.(8)

In clinical practice, the critical question is to differentiate between patients who will benefit from particular therapies. By using molecular assays, more patients can be spared adjuvant chemotherapy. Therefore, surrogate subgroups based on pathological morphology and widely available immunohistochemical (IHC) markers are used for risk stratification and guidance of adjuvant therapy.

ULTRASONOGRAPHY OF BREAST:

Breast ultrasonography is now an essential modality in breast imaging. Ultrasound is indicated as a primary imaging modality in young women; it is used as an adjunct to mammography to evaluate mammographically detected lesions, palpable abnormalities, and other breast symptoms, and after breast MRI as a targeted second-look examination. There are many advanced techniques currently used, like 3D US and sono-elastography. In order to perform state-of-the-art breast ultrasound examinations, it is mandatory to select the proper equipment and transducer, use the appropriate imaging technique, and understand normal sonographic breast anatomy, as well as the sonographic features of benign and malignant breast lesions. High frequency (up to frequency of 18 MHz) for a linear transducer is used for breast imaging.

Grayscale or brightness (B)-mode sonographic features are the basis for the interpretation of breast lesions. Vascularization of breast lesions can be evaluated by color and power Doppler using high Doppler frequencies (7 MHz or more) to visualize vessels within the lesions and to differentiate benign and malignant patterns; however, grayscale morphology is much more important than Doppler in the differentiation of benign and malignant breast lesions. Time-gain compensation is used to achieve homogeneous brightness and image intensity at different breast tissue depths. The gain settings can be adjusted according to the echogenicity of the fatty tissue.

The three main anatomical zones of the breast are the pre-mammary, mammary, and retro-mammary zones, which consist of fatty, fibro glandular, and muscular tissue. Breast fat and fibroglandular tissue lie between the superficial fascia immediately deep to the skin and the deep fascial layer superficial to the pectoralis muscle.

From a sonographic point of view, the breast is divided into various hypoechoic and hyperechoic layers :

Skin (hyperechoic, white fibrous bands)

Fat (hypoechoic, subcutaneous fat lobules)

Breast parenchyma (hyperechoic, fibro glandular soft tissue)

Retromammary fat (hypoechoic fat lobules)

Pectoralis major muscle (hyperechoic fibrous tissue)

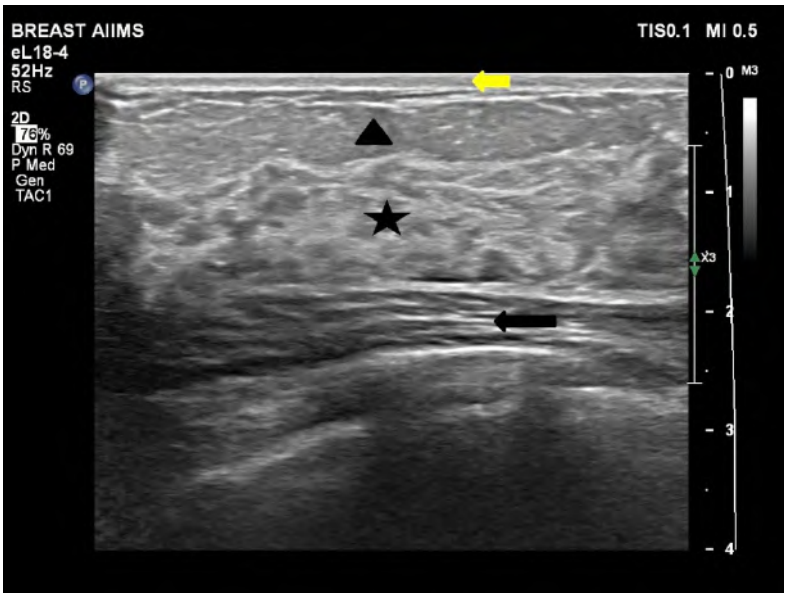


Figure 5: Gray scale USG breast done using 15 MHz linear probe showing main anatomical zones of the breast. Yellow arrow represents the skin surface. Black arrow represents the fatty tissue, star representing the interspersed hyperechoic fibroglandular tissue and black arrow representing the pectoralis major muscle. Underlying normal lung parenchyma is also noted.

MASS ASSOCIATED FEATURES

- Shape (round, oval or irregular)
- Orientation (parallel/anti-parallel)
- Margin (circumscribed/ non circumscribed)
- Echo pattern of lesion (anechoic /hyperechoic/ complex cystic and solid / hypoechoic/isoechoic /heterogeneous)
- Posterior features (no posterior features/enhancement/shadowing/combined pattern)
- Calcifications
- Associated features (architectural distortion/ duct changes/ skin changes/oedema/ vascularity/ elasticity)

Axillary Lymphadenopathy: Present/Absent

- If present, then,
- Single/Multiple
- Ipsilateral/ contralateral
- Cortical thickness

- Hilum
- Extra Nodal Extension

STRENGTHS OF ULTRASOUND AS A MODALITY IN EVALUATION OF BREAST CARCINOMA:

Ultrasound has a high diagnostic yield in differentiating breast cancer from benign lesions and in the detection of early cancers that are mammographically and clinically occult. With higher breast density and in young women sonography outscores mammography where the sensitivity of mammography is relatively low. Usually women younger than 50 years have heterogeneously dense (50–75%) or very thick (> 75%) glandular breast tissue. Women older than 50 years may also have dense breasts, and the sensitivity of mammography in such cases is as low as 30–48% (11). Also, ultrasonography is free of radiation hazards, which allows short interval follow-up and repeated examinations if needed. Ultrasonography is excellent in evaluating the echogenicity of lesions, ducts (dilated, if any) and posterior acoustic features. According to a study, there was no significant difference in sensitivity of US among age groups (P=.790) or breast density groups (P=.526). Still, sensitivity increased significantly in patients with larger tumor sizes (92.9% vs 83.7%, P=.002) and patients with positive lymph nodes (97.6% vs 86.7%, P=.001). (12)US had higher sensitivity than mammography (P<.001), and the combination of MG + US exhibited better sensitivity than MG or US alone and showed significant differences (P<.001). (12)

LIMITATIONS OF ULTRASOUND AS A MODALITY IN EVALUATION OF BREAST CARCINOMA:

Ultrasound has a limited role in the evaluation of the presence of calcifications. Characterization of types of suspicious calcifications cannot be done using ultrasonography. The modality is operator-dependent, and adjustment of various parameters requires experience.

MAMMOGRAPHY OF BREAST:

Initially, traditional film-based radiographic techniques were used to make mammograms. However, film mammography has been replaced by phosphor storage technology or direct digital examination. The phosphor storage systems used a crystal-based technology for transforming X-rays into visible light. Later, direct digital capture systems were developed, utilizing a CCD array that can directly transfer the X-ray into a digital image. Many advantages to these newer technologies compared to film mammography were found, most notably, the dynamic range and contrast resolution that can be adjusted after image capture, allowing radiologists to assess subtle differences within tissues better.

Digital breast tomosynthesis (DBT), also called "3D mammography " has recently become omnipresent. It is performed by obtaining multiple mammographic projections per view, serially acquired along an arc, which are reconstructed into a series of stacked images. The image stacks allow assessment of breast tissue in the X, Y, and Z dimensions decreasing chances of superimposition. (13) This has increased cancer detection rates. Furthermore, this has increased sensitivity in identifying lower-grade cancers. The use of DBT in screening as well as diagnostic mammography has become the standard of care.

The essential components of mammography include an x-ray generator, an image detector and breast compression paddle. Mammography is used to assess the breast on the basis of differential attenuation characteristics of the tissues. The fat attenuates fewer X-rays than fibro glandular tissue and stromal elements and appears grey on mammography. Dense deposits can be identified with better accuracy, such as skin calcifications or calcifications within a neoplastic lesion.

Superimposition may obscure a small mass and this poses a challenge in mammography. To check this, screening mammograms are performed in at least two standard views - the craniocaudal (CC) and the mediolateral oblique (MLO). By comparing these two viewpoints, superimposed structures may be separated. If uncertain, additional diagnostic views with spot compression paddles may be obtained. Also, newer technologies - tomosynthesis imaging allow the radiologist to scroll through the breast in slices As a rule of thumb, the MLO view is inadequate if the pectoralis is not intersected by an orthogonally drawn from the nipple posteriorly, also known as the posterior-nipple line (PNL). The CC view is only adequate if the PNL is within 1 cm of the MLO posterior-nipple line.

A typical mammogram depicts the skin surface, the nipple, the primary breast ducts, fibroglandular tissue, veins, adipose tissue, muscle, and occasionally lymph nodes. (4) The pectoralis has a striated appearance on the MLO view that is denser than the overlying fatty and glandular tissue.

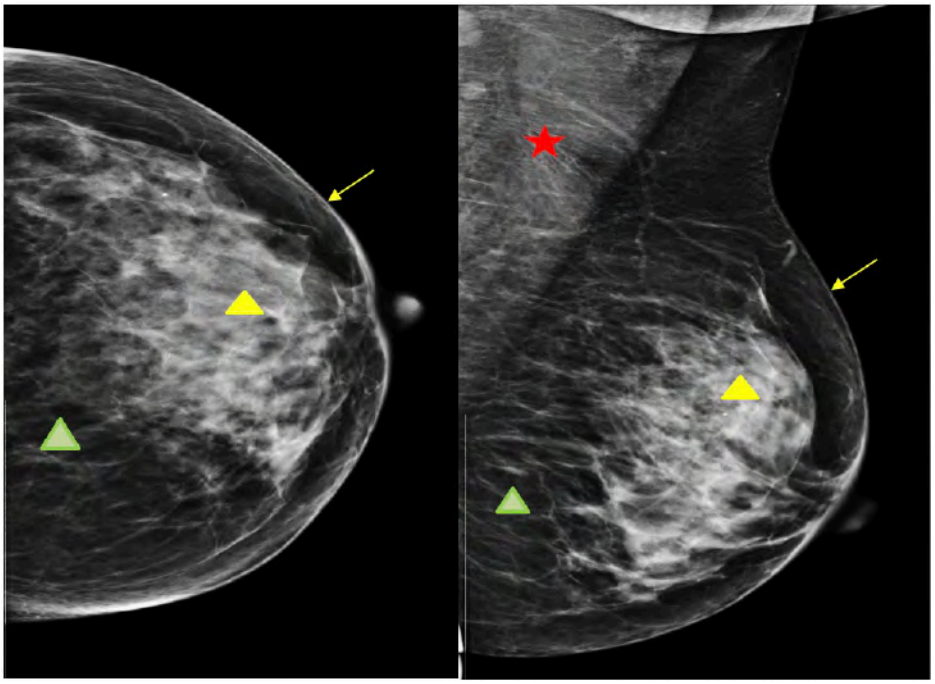


Figure 6 : CC (a) and MLO (b) views of the right breast representing normal components of breast on Mammography. Yellow arrow represents the skin surface. Yellow arrowhead represents the fibroglandular tissue, green arrowhead represents the tissue retro-mammary fatty tissue and red star represents the pectoralis major muscle.

It includes the following parameters:

Breast composition:

A. The breasts are almost entirely fatty

B. There are scattered areas of fibroglandular density

C. The breasts are heterogeneously dense, which may obscure small masses

D. The breasts are incredibly dense, which lowers the sensitivity of mammography

Mass:

- Size (APXTSXCC diameter)
- Shape (round, oval or irregular)
- Margin (circumscribed/obscured/micro lobulated/indistinct/spiculated)
- Location of lesion (laterality, clock face, depth, distance from NAC)

- Density (high/equal/low/fat-containing)
- Calcifications: Suspicious morphology (Amorphous/Coarse heterogeneous/Fine pleomorphic/Fine linear or Fine linear branching) and distribution of calcifications (Diffuse/Regional/Grouped/Linear/Segmental)
- Architectural distortion: Present/Not present and location
- Asymmetry (global/focal/developing)
- Associated features (skin retraction/nipple retraction/skin thickening/trabecular thickening)

Non-Mass associated:

- Calcifications: Suspicious morphology (Amorphous/Coarse heterogeneous/Fine pleomorphic/Fine linear or Fine linear branching) and location of calcification (Diffuse/Regional/Grouped/Linear/Segmental)
- Architectural distortion
- Asymmetry

STRENGTHS OF MAMMOGRAPHY AS A MODALITY IN EVALUATION OF BREAST CARCINOMA:

Mammography is helpful in the evaluation of the calcifications associated with masses. With the use of digital breast tomosynthesis (DBT), the assessment of the margins of the lesions can be done more accurately. The evaluation of non-mass-associated features like focal asymmetry and architectural distortion is better done through mammographic evaluation. Evaluation of ductal carcinoma in situ in the form of segmental/linear distribution of the calcifications can be assessed on mammography. The sensitivity of mammography was significantly greater in patients >50 years (81.3% vs 62.7%, $P<.001$), patients with low breast density (84.5% vs 65.8%, $P<.001$), patients with tumor size >1.0 cm (78.7% vs 65.2%, $P=.001$), and patients with positive lymph nodes (85.7% vs 69.6%, $P=.003$). (12)

LIMITATIONS OF MAMMOGRAPHY AS A MODALITY IN EVALUATION OF BREAST CARCINOMA:

Dense breasts preclude the visualization of mass/abnormality in breast tissue. In the case of locally advanced breast carcinoma, imaging on mammography becomes difficult because the breast cannot be adequately compressed.

BIRADS (Breast Imaging-Reporting and Data System)

BI-RADS is a risk assessment developed by the American College of Radiology (14) that provides a widely accepted lexicon and reporting schema for breast imaging. It applies to mammography, ultrasound, and MRI. BI-RADS provides standardized terminology to describe breast imaging findings.

Table 1: BIRADS categories for reporting and risk prediction of breast lesions.

Assessment	Management	Likelihood of Cancer
Category 0: Incomplete – Need Additional Imaging Evaluation and/or Prior Mammograms for Comparison	Recall for additional imaging and/or comparison with prior examination(s)	N/A
Category 1: Negative	Routine mammography screening	Essentially 0% likelihood of malignancy
Category 2: Benign	Routine mammography screening	Essentially 0% likelihood of malignancy
Category 3: Probably Benign	Short-interval (6-month) follow-up or continued surveillance mammography (Figure 155, see page 152)	> 0% but ≤ 2% likelihood of malignancy
Category 4: Suspicious	Tissue diagnosis	> 2% but < 95% likelihood of malignancy
Category 4A: *Low suspicion* for malignancy		> 2% to ≤ 10% likelihood of malignancy
Category 4B: *Moderate suspicion* for malignancy		> 10% to ≤ 50% likelihood of malignancy
Category 4C: *High suspicion* for malignancy		> 50% to < 95% likelihood of malignancy
Category 5: Highly Suggestive of Malignancy	Tissue diagnosis	≥ 95% likelihood of malignancy
Category 6: Known Biopsy-Proven Malignancy	Surgical excision when clinically appropriate	N/A

HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

Histopathology and immunohistochemistry play an essential role in detecting biomarker expression in breast pathology, and they have demonstrated a strong correlation between receptor expression and patients' responses to therapy. Gene profile analysis is essential but expensive and difficult to perform routinely.

The histological grading of Infiltrating Ductal Carcinoma is done using Nottingham modification of the Bloom-Richardson system, based on (a) tumour tubule formation, (b) several mitotic figures in most active areas and (c) nuclear pleomorphism.

The baseline tool to estimate the likely prognosis of patients with breast cancer is the AJCC staging system, which includes grading, immunohistochemistry biomarkers, and anatomical advancement of the disease. Since its inception in 1977, the American Joint Committee on Cancer (AJCC) has published an internationally accepted staging system based on anatomic findings: tumour size (T), nodal status (N), and metastases (M). However, gene expression profiling has identified several molecular subtypes of breast cancer. The 8th edition of the AJCC staging manual (2018) gives a new prognostic staging system for breast cancer that acknowledges biological factors in addition to anatomical features [15]. These factors—ER, PR, HER2, grade, and multigene assays—are recommended in practice to define prognosis.

The most widely used histologic grading system for breast cancer is the Elston-Ellis modification of the Bloom-Richardson grading system, also known as the Nottingham grading system. The grade of a tumor is determined by assessing morphologic features: (a) formation of tubules, (b) mitotic count, (c) variability, and the size and shape of cellular nuclei. A score between 1 (most favorable) and 3 (least favorable) is assigned for each feature. Grade 1 corresponds to combined scores between 3 and 5, grade 2 corresponds to a combined score of 6 or 7, grade 3 corresponds to a combined score of 8 or 9.

HISTOLOGICAL GRADING

We shall use Nottingham modification of Bloom-Richardson system, based on:

(a) tumor tubule formation,

(b) number of mitotic figures in most active areas and

(c) nuclear pleomorphism.

Table 2: Nottingham Modification of Bloom Richardson system for assessment of histological grading of breast lesions

Nottingham modification of Bloom-Richardson system	**Points Allocated**	
Tumor tubule formation	1 point	> 75% of tumor
	2 points	10 - 75% of tumor
	3 points	< 10% of tumor

Number of mitotic figures in most active areas	Points	(a)	(b)	(c)
	1 point	0-5	0-9	0-11
	2 points	6-11	10-19	12-22
	3 points	11+	20+	23+

Nuclear pleomorphism	Points	
	1 point	minimal nuclear variation in size and shape; small regular uniform cells
	2 points	moderate nuclear variation in size and shape
	3 points	marked nuclear variation in size and shape

Final scoring is made after adding all above:

3 - 5 points: well differentiated (grade I)

6 - 7 points; moderately differentiated (grade II)

8 - 9 points: poorly differentiated (grade III)

ROLE OF IMMUNOHISTOCHEMISTRY

Invasive breast cancer with no special type (NST), formerly known as invasive ductal carcinoma is the most frequent subgroup (40–80%). (16) It is divided into various histological special types(17) . About 25% of invasive breast cancers present distinctive growth patterns and cytological features, hence, they are recognized as specific subtypes (e.g., invasive lobular carcinoma, tubular, mucinous A, mucinous B, neuroendocrine). The classification is based on the molecular receptors as described below:

Estrogen receptor (ER) is an important diagnostic determinant since approximately 70–75% of invasive breast carcinomas are characterized by significantly enhanced ER expression. (18) Measurement of ER expression is mandatory to provide the selection of those patients who will most benefit from the implementation of the endocrine therapy, mainly selective estrogen receptor modulators, pure estrogen receptor down regulators, or third-generation aromatase inhibitors. (7) ER expression might also constitute a

predictive factor—patients with high ER expression usually present significantly better clinical outcomes.(19)

PR is highly expressed (>50%) in patients with ER-positive while quite rarely in those with ER-negative breast cancer. (20) The ER regulates PR Expression; therefore, the physiological values of PR inform the functional ER pathway (20). However, both ER and PR are abundantly expressed in breast cancer cells, and both are considered diagnostic and prognostic biomarkers of breast cancer (especially ER-positive ones). (21) Greater PR expression is positively associated with overall survival, time to recurrence, and time to either treatment failure or progression, while lowered PR levels are usually related to a more aggressive course of the disease and poorer recurrence and prognosis. (22) Thus, the favourable management of breast cancer patients depends highly on assessing PR expression.

Expression of the HER2 accounts for approximately 15–25% of breast cancers, and its status is primarily relevant in the choice of proper management with breast cancer patients; HER2 overexpression is one of the earliest events during breast carcinogenesis. (23) Overexpression of HER2 also correlates with a significantly shorter disease-free period as well as histologic type, pathologic state of cancer, and several axillary nodes with metastatic cancerous cells. (24)

The Ki-67 is a marker of cell division, and the Ki-67 proliferation index is an excellent marker to provide information about the proliferation of cancerous cells, particularly in the case of breast cancer. The proliferative activities determined by Ki-67 reflect the aggressiveness of cancer, the response to treatment, and the recurrence time. Thus, Ki-67 is crucial in choosing the proper treatment therapy and the potential follow-ups due to recurrence.(25) High expression of Ki-67 also reflects poorer survival rates in breast cancer patients.

Independent of histological subtypes of invasive breast cancer, molecular classification is divided into Luminal A, Luminal B, HER2-enriched and TNBC.

Luminal A tumors are characterized by the presence of estrogen-receptor (ER), progesterone-receptor (PR), and the absence of HER2. In this subtype, the ER transcription factors activate genes, the expression of which is characteristic of luminal epithelium lining the mammary ducts [18,19]. It also presents a low expression of genes related to cell proliferation [26]. Clinically, they are low-grade, slow-growing, and tend to have the best prognosis.

Luminal B tumors are higher grade and have a worse prognosis. They are ER-positive

and maybe PR negative and HER2 positive. This subtype has lower expression of genes or proteins typical for luminal epithelium, such as the PR. (27) ER is similarly expressed in both A and B subtypes and distinguishes luminal from non-luminal disease. (28)

The HER2-enriched group makes up 10–15% of breast cancers. It is characterized by the high expression of the HER2 with the absence of ER and PR. HER2-enriched cancers grow faster than luminal cancers and used to have the worst prognosis of subtypes before the introduction of HER2-targeted therapies. Notably, the HER2-enriched subtype is not synonymous with clinically HER2-positive breast cancer because many ER-positive/HER2-positive tumours qualify for the luminal B group. Moreover, about 30% of HER2-enriched tumours are classified as clinically HER2-negative based on immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) methods. (28)

Triple-negative breast Cancer (TNBC) is a heterogeneous collection of breast cancers characterized as ER-negative, PR-negative, and HER2-negative. They constitute about 20% of all breast cancers. TNBC is more common among women younger than 40 years of age. TNBC tends to be biologically aggressive and is often associated with a worse prognosis. (29)

PRIOR STUDIES IN LITERATURE ABOUT IMAGING APPEARANCES OF VARIOUS MOLECULAR SUB-TYPES

En Sook Ko et al. (2010) found that Triple-negative breast cancers showed a high histological grade. On mammography, triple-negative breast cancers usually presented with a mass (43/87, 49%) or focal asymmetry (19/87, 22%) and were less associated with calcifications. On ultrasound, the cancers were less frequently seen as non-mass lesions (12/87, 14%), more likely to have circumscribed margins (43/75, 57%), were markedly hypoechoic (36/75, 57%) and less likely to show posterior shadowing (4/75, 5%). Among the three types of breast cancers, ER-negative/PR-negative/HER2-positive breast cancers most commonly had associated calcifications (52/65, 79%) on mammography and were depicted as non-mass lesions (21/65, 32%) on ultrasound.(30)

Chun Yan-Li et al. (2013) studied among Chinese women. They found that TNBC is associated with younger disease onset age, larger tumour size, higher axillary lymph node positivity rate, and higher tumour histologic grade. TNBC subtype was also found

to be related to a family history of breast cancer, increased metastatic risk and poor prognosis. (31)

Park et al. (2014), in a study on Korean women, found a positive correlation between the occurrence of HER2 neu-positive breast carcinoma and higher breast density. (6)

Elias et al (2014) studied the presence of microcalcifications on mammography [pooled odds ratio (p OR), 3.14; 2.46-4.00] and ultrasound (mass-associated p OR, 2.95) and found that fine linear branching or fine linear microcalcifications (p OR, 2.11; 1.07-4.14) or extremely dense breasts on mammography (p OR, 1.37; 1.07-1.76), (p OR, 1.57; 1.11-2.21) - all were associated with increased the chance of HER2 overexpression. (32)

Anupama *et al. (2017)* found that circumscribed margins, posterior enhancement, and large size correlated with triple-negative BC (TNBC), while high-risk microcalcifications and micro-lobulated margins predicted HER2-enriched cancers. At the same time, luminal subtypes tend to be small-sized lesions with spiculated margins and posterior acoustic shadowing (Luminal A type). In addition, they also found that ultrasound size signals a higher odds ratio for TNBC if presented during screening. As TNBC usually displays sonographic features such as circumscribed margins and posterior enhancement, this causes visual similarity with benign common lesions decreasing threshold to advice biopsy .(33)

Rashmi et al. (2018) found that tumors with non-circumscribed margins and posterior acoustic shadowing were more likely to be luminal A or B subtype [odds ratio (OR) 5.78; 95% confidence interval (CI) 3.68–9.80; $P < 0.0001$]. Tumors having non-circumscribed margins and posterior acoustic shadowing with high vascularity were more commonly found to be luminal B subtypes (OR 2.88; 95% CI 2–4.14; P-<0.0001). Tumors with microcalcification and posterior mixed acoustic pattern were strongly associated with HER2-positive (OR 5.48; 95% CI 3.06–9.80; $P < 0.0001$). Tumors with circumscribed margins and posterior acoustic enhancement were highly suggestive of triple-negative breast cancer (OR 7.06; 95% CI 4.64–10.73; $P < 0.0001$).(34)

Tay Wei Ming et al. (2021) found that high-risk microcalcifications and micro-lobulated margins predicted HER2-enriched cancers. High-risk microcalcifications were found in slightly under one-third of the total sample ($n = 105$, 32%) but were more prevalent amongst BC patients with the subtype HER2-enriched ($n = 24$, 48%). Posterior acoustic enhancement was more common in HER2-enriched tumours ($n = 16$,

32%) and TNBC (*n* = 33, 69%). (35)

Sahar et al. (2023) found that six features were suggestive of HER-enriched carcinoma on mammogram and ultrasound: (1) irregular shape mass, (2) indistinct margin, (3) associate fine pleomorphic microcalcifications, (4) asymmetry of global distribution, (5) hypoechoic pattern on ultrasound and (6) enlarged pathological nodes. There was a significant correlation between the HER2-enriched subtype and the presence of fine pleomorphic calcifications ($p = < 0.02$) of segmental distribution ($p = < 0.002$) and asymmetry of global distribution ($p = < 0.001$). On ultrasound, pathological lymphadenopathy of the malignant pattern was noted in luminal B, while reactionary-looking or non-specific axillary nodes were the typical pattern for the HER2-enriched subtype. (36)

Portnow et al. (2023) showed that irregular hypoechoic masses with echogenic foci of microcalcifications in the US have also been shown to be associated with HER2-positive overexpression. (37)

Jennifer et al (2023) found that TNBCs can manifest with imaging features that include circumscribed margins, parallel orientation, hypoechoic to nearly anechoic internal echogenicity, and complex cystic and solid masses with posterior acoustic enhancement. These features can result in the misinterpretation of TNBCs as benign cysts, fibroadenomas, or abscesses. (38)

Brahmi et al. (2024) found that mammograms' micro-lobulated margin and oval/round morphology correlate with triple-negative intrinsic subtype ($P = 0.006$ and $P = 0.004$). Calcification in sonography was significantly higher in the luminal-B subtype ($P = 0.002$). Ill-defined margins in mammography were substantially higher in amplified HER2 expression ($P = 0.004$) in the same manner as an oval/round shape in higher levels of Ki-67 ($P = 0.030$).(39)

IHC panels for every patient are sometimes not feasible due to resource limitations, especially in third-world countries like ours. This led us to consider whether routine imaging modalities like mammography and ultrasound can determine and predict the hormonal status of the tumor. Also, some patients with the TNBC molecular subtypes need earlier treatment as this subtype is prone to metastasize earlier than other subtypes. To our knowledge, studies with combined assessment of patients with sono-mammography are yet to be done in Central India. This study is an attempt to analyse the imaging features of breast masses that come to our institute.

AIM:

To establish a correlation between Imaging features of Infiltrating Breast Carcinoma on Mammography and Ultrasound modalities with the molecular subtypes (Luminal A, Luminal B, HER2neu enriched and TNBC) and to compare their performance in predicting molecular subtypes.

OBJECTIVES:

To establish a correlation between tumoral and peri-tumoral imaging features of histopathologically proven Infiltrating Breast Carcinoma on mammography and ultrasound with the molecular subtypes (Luminal A, Luminal B, HER2neu enriched and TNBC).

To compare the performance of Mammography and Ultrasound modalities, individually and in conjunction, in predicting the molecular subtypes (Luminal A, Luminal B, HER2neu enriched and TNBC) using imaging features.

MATERIAL AND METHODS:

Our study was a mammography and ultrasound-based cross-sectional study. The study was undertaken after due approval from the Institutional Ethics Committee for post-graduate research (IHEC- PGR) of AIIMS Bhopal. The study was conducted in the Department of Radiodiagnosis and Imaging, All India Institute of Medical Sciences, Bhopal, Madhya Pradesh, India, for a period of 18 months after getting necessary approvals from the Thesis Review Committee and Institutional Human Ethics Committee, AIIMS Bhopal (from May 2023 to October 2024).

All histopathologically proven cases of Infiltrating Breast Carcinoma with known IHC marker status who were referred to the Department of Radiodiagnosis and Imaging for further evaluation were included in the study.

Written informed consent was obtained from every patient/relative before recruitment (consent form enclosed).

INCLUSION CRITERIA: Histopathologically proven cases of Infiltrating Breast Carcinoma with known IHC marker status who underwent imaging in the Department of Radiodiagnosis and Imaging, AIIMS Bhopal.

EXCLUSION CRITERIA:

1. Patients who are not histopathologically proven cases of Infiltrating Breast Carcinoma.

2. Patients whose IHC marker status has yet to be discovered.
3. Patients who have been previously treated for breast cancer.

SAMPLE SIZE: The sample size for this study was calculated based on another survey https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5355144/that reported the relative proportion of Luminal B molecular subtype of infiltrating ductal carcinoma as 22.2%. We presume the hypothesized frequency with precision of 10% and a Design effect of 1. With this, the sample size was calculated to be 115 on a confidence interval of 99%.

Sample Size for Frequency in a Population

Population size (for finite population correction factor or fpc) (*N*):	1000000
Hypothesized % frequency of outcome factor in the population (*p*):	22.2%+/-10
Confidence limits as % of 100(absolute +/- %) (*d*):	10%
Design effect (for cluster surveys-*DEFF*):	1

Sample Size(*n*) for Various Confidence Levels

Confidence Level (%)	**Sample Size**
95%	67
80%	29
90%	47
97%	82
99%	115
99.9%	187
99.99%	262

Equation

Sample size $\boldsymbol{n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1) +p*(1-p)]}$

In our department, about 1157 patients came whose sono-mammogram was conducted. Of these patients who were biopsied/ surgery, 160 met all the inclusion criteria and were included in the study after obtaining written consent.

STUDY DESIGN:

All patients of suspected breast malignancy being referred to

Department of Radiodiagnosis and Imaging, AIIMS Bhopal, for imaging during the period of study

Undergo Mammography and Ultrasound in the Department of Radiodiagnosis And Imaging, AIIMS Bhopal

Excluding:
Tissue diagnosis other than Infiltrating Breast Carcinoma
IHC marker status not known
Previously treated patients.

Patients who are found to be cases of Infiltrating Breast Carcinoma with Known IHC marker status and Histological grade included in the study

Establishing a relationship between tumoral and peri-tumoral imaging features of histopathologically proven Infiltrating Breast Carcinoma on mammography and ultrasound with the molecular subtypes (Luminal A, Luminal B, HER2neu enriched and TNBC).

MATERIAL AND METHODOLOGY:

EQUIPMENT:

Ultrasound examination was done on GE Voluson S8 and Philips Affinity 70 using a Linear probe of frequency 15 MHz using the radial technique, following radial and anti-radial planes, centering the transducer at the nipple, and then stretching outwards, following breast lobes as per anatomy. All detected lesions were visualized in at least two orthogonal planes. A color Doppler examination of the mass lesion followed this.

Subsequently, the contralateral breast and bilateral axilla were examined to evaluate for any other mass and axillary lymph nodal involvement.

Evaluation of breast masses on ultrasonography includes the following parameters:

- Shape (round, oval or irregular)
- Orientation (parallel/anti-parallel)
- Margin (circumscribed/ non circumscribed)
- Echo pattern (anechoic/hyperechoic/complex cystic and solid/hypoechoic/isoechoic/heterogeneous)
- Posterior features (no posterior features/enhancement/shadowing/combined pattern)
- Calcifications
- Associated features (architectural distortion/ duct changes/ skin changes/oedema/ vascularity.

Figure 7: PHILIPS Affinity 70 ultrasound scanner with probe

Figure 8: GE LOGIQ S8 ultrasound scanner with probe

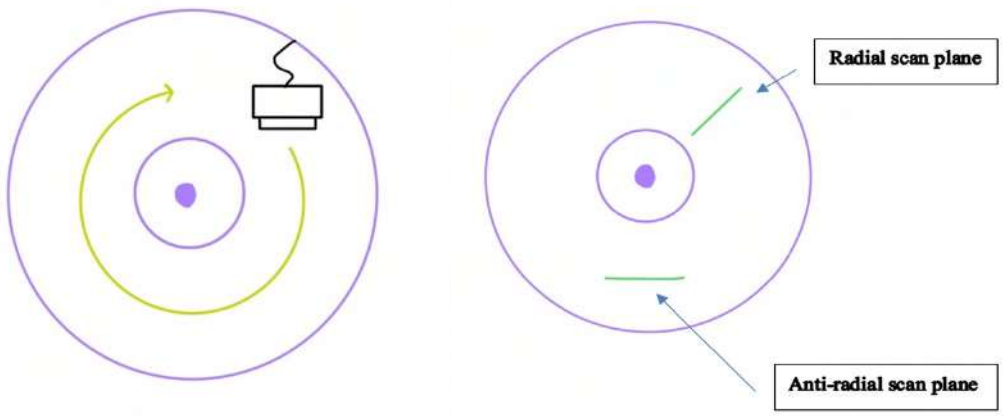


Figure 9: Ultrasound scanning Technique with probe

Mammography was done using 3D Digital mammography Hologic Selenia Dimensions 2D/3D Mammography system.

Figure 10: Hologic Selenia Dimensions 2D/3D Mammography system.

The patients were examined using two standard views in screening mammography, named for the direction of the x-ray beam from the source to the detector: craniocaudal (CC) and mediolateral oblique (MLO).

In many instances, when the lesions' margins were unclear, significant overlapping precluded diagnostic evaluation of the type of calcification and assessed asymmetries, focal compression or magnification paddles were used. The focal compression paddle was placed by estimating the location of the lesion based on the screening mammogram images. Magnification views were performed by lifting the breast away from the image detector, allowing for geometric magnification.

Images obtained were examined using standard parameters as mentioned in ACR-BIRADS.

Breast composition:

(Type-a): The breasts are almost entirely fatty

(Type-b): There are scattered areas of fibroglandular density

(Type-c): The breasts are heterogeneously dense, which may obscure small masses

(Type-d): The breasts are incredibly dense, which lowers the sensitivity of

mammography

Mass:

- Size (APXTSXCC diameter)
- Shape (round, oval or irregular)
- Margin (circumscribed/obscured/micro lobulated/indistinct/spiculated)
- Location of lesion (laterality, clock face, depth, distance from NAC)
- Density (high/equal/low/fat-containing)
- Calcifications: Suspicious morphology (Amorphous/Coarse heterogeneous/Fine pleomorphic/Fine linear or Fine linear branching) and distribution of calcifications (Diffuse/Regional/Grouped/Linear/Segmental)
- Architectural distortion: Present/Not present and location
- Asymmetry (global/focal/developing)
- Associated features (skin retraction/nipple retraction/skin thickening/trabecular thickening**)**

Non-Mass associated:

- Calcifications - Suspicious morphology (Amorphous/Coarse heterogeneous/Fine pleomorphic/Fine linear or Fine linear branching) and location of calcification (Diffuse/Regional/Grouped/Linear/Segmental)
- Architectural distortion
- Asymmetry

HISTOPATHOLOGICAL ANALYSIS AND IMMUNOHISTOCHEMISTRY;

The histological and immunohistochemical study was performed on tissue pieces fixed in a 10% neutral formalin aqueous solution and embedded in paraffin cubes at 55˚C. Histological sections from all samples were observed under a photon microscope. Experienced pathologists assessed immunohistochemical staining. ER and PR were considered positive when >1% of cells showed positive nuclear expression. HER-2 categorization was made as follows- negative (score 0 & score 1+), equivocal (score 2+), positive (score 3+). Equivocal HER2Neu cases underwent FISH analysis and amplification was determined. Regarding Ki-67, we considered low expression when ≤20% and high when >20% of cells showed positive nuclear expression.

Table 3: Receptor types in different molecular subtypes in breast carcinoma.

Luminal A:ER/PR +, HER2neu -, Ki67 <20%
Luminal B:ER/PR +, HER2neu +/-, Ki67 >20%
HER2neu enriched: ER-, PR-, HER2neu +
TNBC: ER-, PR-, HER2neu -

ASSESSMENT OF PARTICIPANTS:

Participants were evaluated on the basis of standard parameters as mentioned in ACR BIRADS Lexicon for Ultrasound and Mammography. All patients were evaluated and due written consent was obtained. Then the patients were followed up for the histopathological and immune-histochemistry evaluation. All patients who met the inclusion criteria were included in the study.

Our study is prospective study in which when any patient with any complaints related to breast was referred to us for sono-mammography, we screened the patient according to standard reporting lexicon by ACR-BIRADS. All the patients whose sono-mammography raised the suspicion of malignancy were asked for consent for participation in the study.

In a period of 18 months, 462 patients were primary cases suspected to have malignancy and they were assessed on mammography and sonography and conferred BIRADS category of 4A/B/C and 5 according to the imaging appearances of the lesion. The patients were followed up for histopathology and IHC reports. Of 462 patients whose imaging findings were suspicious of malignancy, about 315 underwent biopsy at our institution. A total of 223 patients' histopathology samples revealed Infiltrating Ductal Carcinoma. Of the 223 patients, only 174 patients underwent immunohistochemistry evaluation. Of them, 14 patients had equivocal HER2neu status and were advised FISH analysis. However, FISH could not be done and these patients were excluded. Finally, 160 patients satisfied all our inclusion criteria and were included in the study. 6 patients had large masses in their breasts, which precluded characterization of the mass and hence were excluded from statistical analysis. Finally, we achieved a number of 154 patients who met all our inclusion criteria.

The collected data was entered in Microsoft Excel and then analyzed and statistically

evaluated using the SPSS-25 version. The normality of each variable was assessed by using the Kolmogorov- Smirnov test and Shapiro-Wilk test. Quantitative data was expressed by mean, standard deviation and depends on normal distribution, difference between means of two groups were tested by unpaired t test or Mann Whitney U test while for pre-post comparison paired t test or Wilcoxon signed rank test was used. Qualitative data was expressed in percentage. Spearman correlation coefficient was used to see correlation between quantitative variables. ‘p’ value less than 0.05 was considered statistically significant.

OBSERVATIONS AND RESULTS:

Demographic Data:

A total of 154 patients were included in the study, with age of patients ranging from 34-86 years ago with average age of presentation being 52 ±11 years. Of all the molecular subtypes, Triple Negative breast carcinoma (TNBC) shows earliest mean age of presentation while the Luminal A subtype showed latest mean age of presentation. Of all the molecular subtypes, HER2neu enriched subtype had earliest age of menarche while the Luminal A subtype showed latest mean age of menarche.

Table 4: Demography in different molecular subtypes in study subjects (n=154)

	Her2 neu (n=43)	**Luminal-A (n=16)**	**Luminal-B (n=64)**	**TNBC (n=31)**	**Total**
Mean age of presentation	51.91±9.50	55.63±14.03	54.31±12.39	49.10±10.34	52.73±11.54
Menarche age	12.98±1.05	14.19±1.64	13.44±1.48	13.77±1.70	13.45±1.48

Upon statistical analysis, no significant statistical significance was noted with age of presentation and the type of molecular subtype. The p-value for the mean age of presentation was 0.124. Similarly, the p-value for the mean age of menarche was 0.138.

Table number 5 shows the distribution of various molecular subtypes. Most of our patients were of the molecular subtype Luminal B, followed by HER2Neu enriched.

Table 5: Distribution of types of molecular subtypes in study subjects (n=154)

Type of molecular subtype on HISTOPATHOLOGICAL	**No.**	**%**
HER2 neu	43	27.9
Luminal-A	16	10.4

Luminal -B	64	41.6
TNBC	31	20.1

For the histological grading, Nottingham grade was used to evaluate the patients. Our patient data had the following distribution with the most common grade being Grade 2 in about 58.4 % of all patients.

Table 6: Distribution of percentages of Nottingham grade on histopathology in study subjects (n=154)

Nottingham grade	**No.**	**%**
1	27	17.5
2	90	58.4
3	37	24.0

Table 7 shows Nottingham grade in different molecular subtype. 62.5% of patients of the Luminal A subtype presented with grade 1 where, whereas other types were mostly grade 2 or 3.

Table 7: Nottingham grade in different molecular subtype on histopathology in study subjects (n=154)

Grade	**Her2 neu (n=43)**	**Luminal-A (n=16)**	**Luminal-B (n=64)**	**TNBC (n=31)**	**Total**
1	6(14.0%)	10(62.5%)	9(14.1%)	2(6.5%)	27(17.5%)
2	25(58.1%)	6(37.5%)	42(65.6%)	17(54.8%)	90(58.4%)
3	12(27.9%)	0(0.0%)	13(20.3%)	12(38.7%)	37(24.0%)

Table 8 shows clinical features of our patients- almost all our patients presented with a palpable mass, rest 3 (2%) patients presented with only nipple discharge (2 patients

with bloody discharge and 1 with serous discharge).

Table 8: Clinical features of patients in different molecular subtype in study subjects (n=154)

		Her2 neu (n=43)	Luminal-A (n=16)	Luminal-B (n=64)	TNBC (n=31)	Total
	Palpable lump	41(95.3%)	16(100.0%)	63(98.4%)	31(100.0%)	151(98%)
Side	Left	22(51.2%)	9(56.3%)	36(56.3%)	20(64.5%)	87(56.5%)
	Right	21(48.8%)	7(43.8%)	28(43.8%)	11(35.5%)	67(43.5%)
Nipple Characteristics	Nipple deviation	3(7.0%)	4(25.0%)	8(12.5%)	2(6.5%)	17(11.0%)
	Nipple retraction	5(11.6%)	3(18.8%)	19(29.7%)	4(12.9%)	31(20.1%)
	Nipple discharge	5(9.3%)	1(6.3%)	0(0.0%)	0(0.0%)	5(3.2%)
Macroscopic skin changes	Skin changes	7(16.3%)	2(12.5%)	12(18.8%)	4(12.9%)	25(16.2%)
	Skin adherent	5(11.6%)	1(6.3%)	6(9.4%)	3(9.7%)	15(9.7%)
	Signs of inflammation	3(7.0%)	1(6.3%)	1(1.6%)	1(3.2%)	6(3.9%)
	Peau'd orange	2(4.7%)	1(6.3%)	0(0.0%)	0(0.0%)	3(1.9%)
Clinically palpable Axillary lymph node	Absent	32(74.4%)	12(75.0%)	33(51.6%)	20(64.5%)	97(63.0%)
	Present	11(25.6%)	4(25.0%)	31(48.4%)	11(35.5%)	57(37.0%)
No.	Single	8(18.6%)	2(12.5%)	20(31.3%)	9(29.0%)	39(25.3%)
	Multiple	3(7.0%)	2(12.5%)	11(17.2%)	2(6.5%)	18(11.7%)
side	Ipsilateral	11(25.6%)	4(25.0%)	31(48.4%)	11(35.5%)	57(37.0%)
	Contralateral	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)

In the case of clinically palpable lumps, most patients presented with lump in the upper

outer quadrant of breast (UOQ), followed by central, upper inner quadrant, lower outer quadrant and lower inner quadrant.

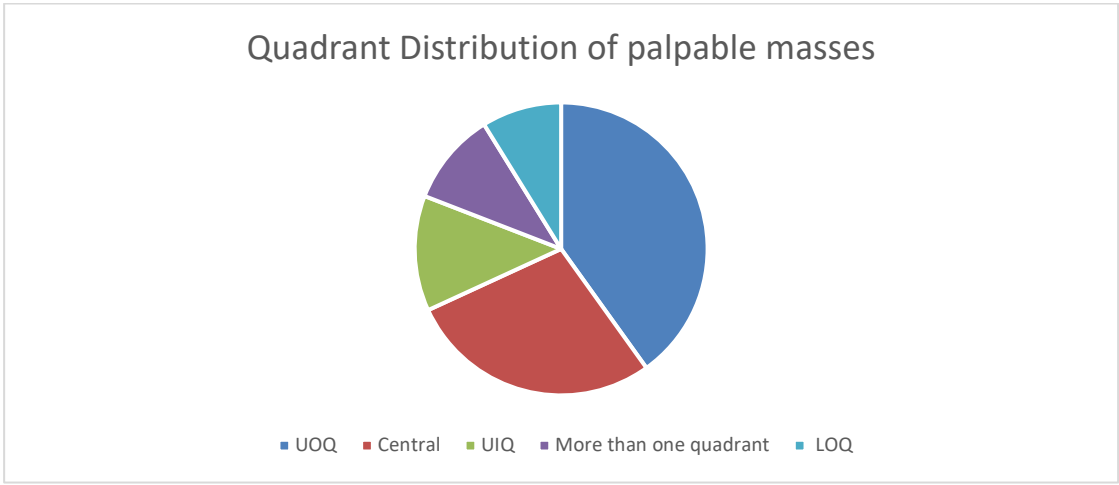


Figure 11: Quadrant distribution of the palpable masses in study subjects (n-142)

Of the 154 patients, 75 patients had complaints in the left breast, while the rest had complaints on the right breast.

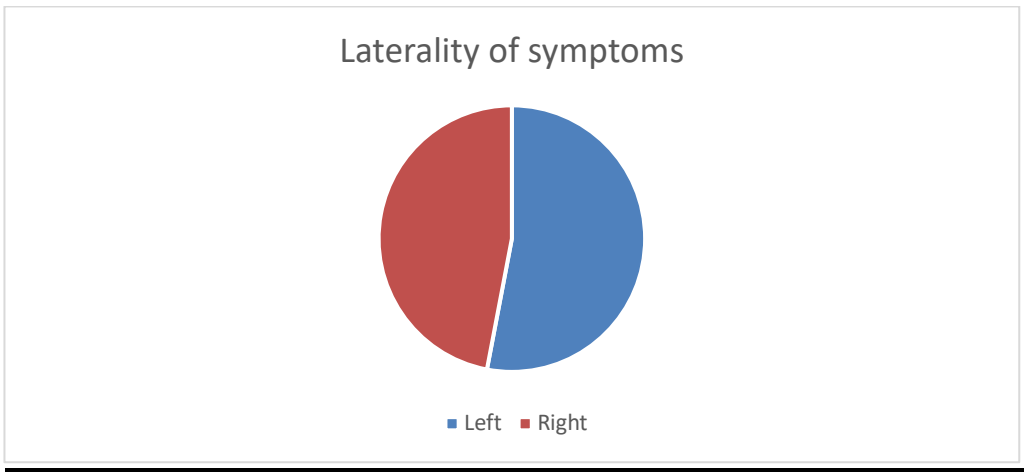


Figure 12: Side distribution of the palpable masses in study subjects.

MAMMOGRAPHIC BREAST DENSITY

Out of 154 patients, the majority of patients had Type B and Type C breasts. Luminal B, and TNBC molecular subtypes occurred in higher breast density- Type C. Luminal A and HER2Neu enriched subtype, however, was noted in Type B breast followed by Type C.

Table 9: Types of breasts according to mammographic density in different molecular subtype in study subjects (n=154)

		Her2 neu (n=43)	Luminal-A (n=16)	Luminal-B (n=62)	TNBC (n=33)	Total (n=154)
Type of breast	A	0(0.0%)	2(12.5%)	0(0.0%)	0(0.0%)	2(1.3%)
	B	19(44.2%)	9(56.3%)	30(46.9%)	12(38.7%)	70(45.5%)
	C	18(41.9%)	5(31.3%)	30(46.9%)	17(54.8%)	70(45.5%)
	D	6(14.0%)	0(0.0%)	4(6.3%)	2(6.5%)	12(7.8%)

EVALUATION OF THE MAMMOGRAPHIC FEATURES OF DIFFERENT MOLECULAR SUBTYPES

Among 154 patients, 12 patients had masses that were no seen on mammography. 6 patients were HER2Neu enriched, 2 were Luminal A patients and 3 patients were Luminal B subtype and 1 was TNBC subtype.

SIZE:

Out of 142 patients, most of the masses were in range of size 2.1-5 cm (65.4%).16.9% of masses had a size range of 1.1-2 cm. Only 17.5% of patients had masses of size more than 5 cm in maximum dimension. TNBC subtype patients had large masses on presentation.

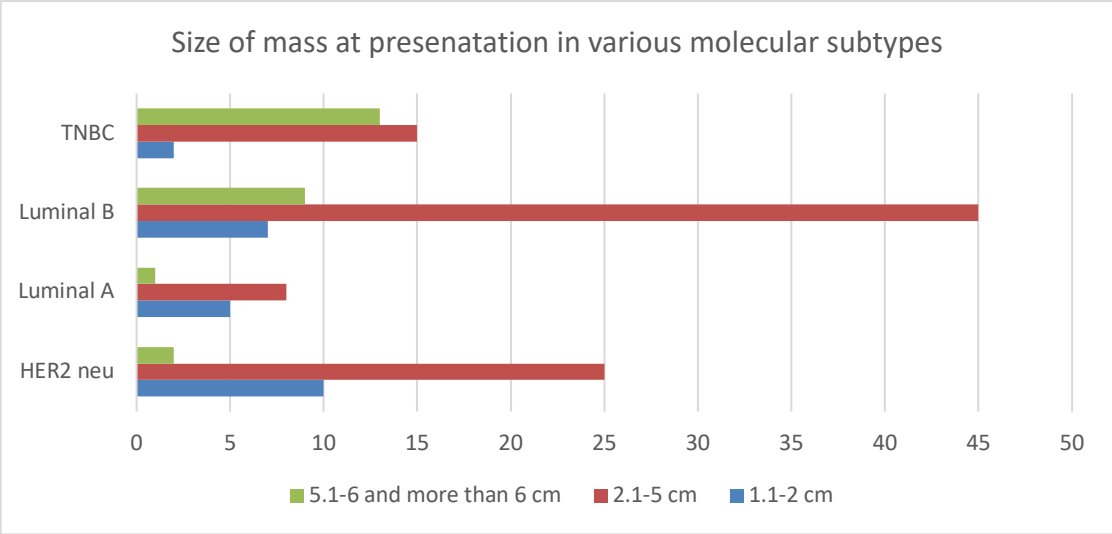


Figure13: Frequency distribution of Size of the palpable masses in study subjects (n-142).

SHAPE:

Out of 142 patients, 97.1% patients had breast masses that were irregular in shape. Irregular shape was most commonly noted in Luminal A (100%), followed by Luminal B subtype (96.7%), followed by HER2neu enriched (94.5%). The TNBC subtype had irregular shape with micro-lobulations. About 10% of the TNBC subtype patients had well circumscribed round or oval shaped masses.

Table 10: Types of shapes of masses in different molecular subtypes in study subjects (n=142).

Shape	**HER2 Neu (n=36)**	**Luminal A (n=14)**	**Luminal B (n=61)**	**TNBC (n=30)**	**Total (n=142)**
Irregular	35(94.5%)	14(100%)	59(96.7%)	27(90%)	135(97.1%)
Oval	1(2.75%)	0(0.0%)	0(0.0%)	2(6.6%)	3(2.1%)
Round	1(2.75%)	0(0.0%)	2(3.3%)	1(3.3%)	4(2.8%)

MARGINS:

Most predominant margin

Of our 142 patients, most predominant margin in the breast masses was micro-lobulated margin (64.7%) in all subtypes followed by spiculated margins (25.3%).

In the TNBC subtype of patients, most patients had micro lobulated margins (78.1%)

and circumscribed margins (20%). Only 6.2% patients had spiculations in few of the masses.

In HER2Neu-enriched subtype patients, most patients had micro-lobulated margins (67.5%), and about 21.6% of patients had spiculated margins. Only 8% patients showed circumscribed margins.

In Luminal A subtype, 57.1% patients had spiculated margins and 42.8% patients had micro lobulated margins. None of the patients showed indistinct or circumscribed margins.

In the Luminal B subtype, micro lobulated (58%) and spiculated margins (29%) were most common. 8% of patients had an indistinct margin as the predominant margin.

Table 11: Types of margins of masses in different molecular subtype in study subjects (n=142)

		Her2 neu (n=43)	Luminal-A (n=16)	Luminal-B (n=62)	TNBC (n=33)	Total (n=142)
Most predominant margin	Circumscribed	3(8.1%)	0(0.0%)	0(0.0%)	5(20%)	8(5.6%)
	Indistinct	1(2.7%)	0(0.0%)	5(8%)	0(0.0%)	6(4.2%)
	Microlobulated	25(67.5%)	6(42.8%)	36(58%)	25(78.1%)	92(64.7%)
	Spiculated	8(21.6%)	8(57.1%)	18(29%)	2(6.2%)	36(25.3%)

Out of 142 patients, 36 (25.3%) showed spiculated margin. Least was TNBC subtype-only 6% patients showed spiculated margins.

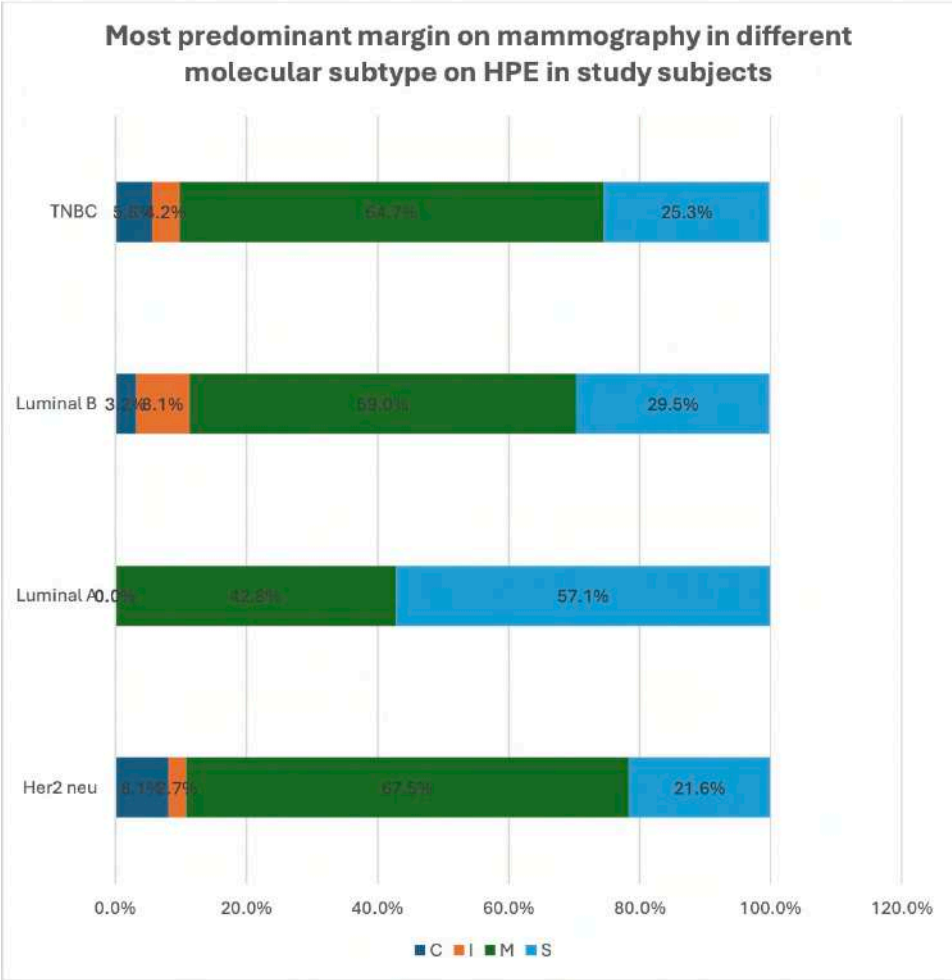


Figure 15: Percentage distribution of type of uncircumscribed margins in masses in different molecular subtype (n=142). (C- circumscribed, M-micro-lobulated, I-indistinct and S- spiculated).

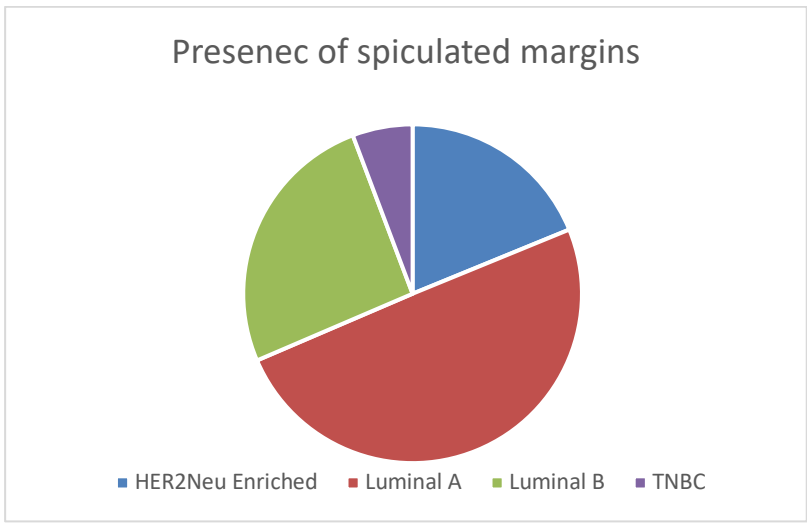


Figure 16: Percentage distribution of spiculated type of margins in masses in

different molecular subtypes in study subjects (n=142)

MAMMOGRAPHIC DENSITY OF LESIONS:

Most masses were iso-dense to the normal surrounding fibroglandular tissue. A small percentage of patients had hyperdense mass on mammography- most were Luminal B molecular subtype.

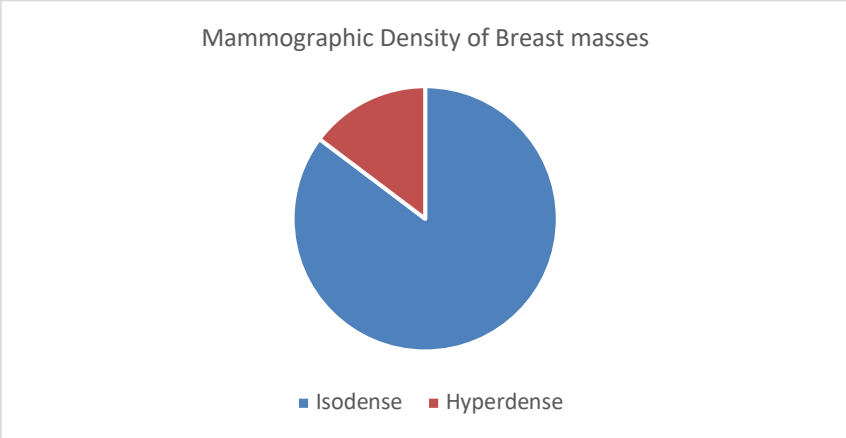


Figure 17: Percentage distribution of micro lobulated type of margins in masses in study subjects (n=142)

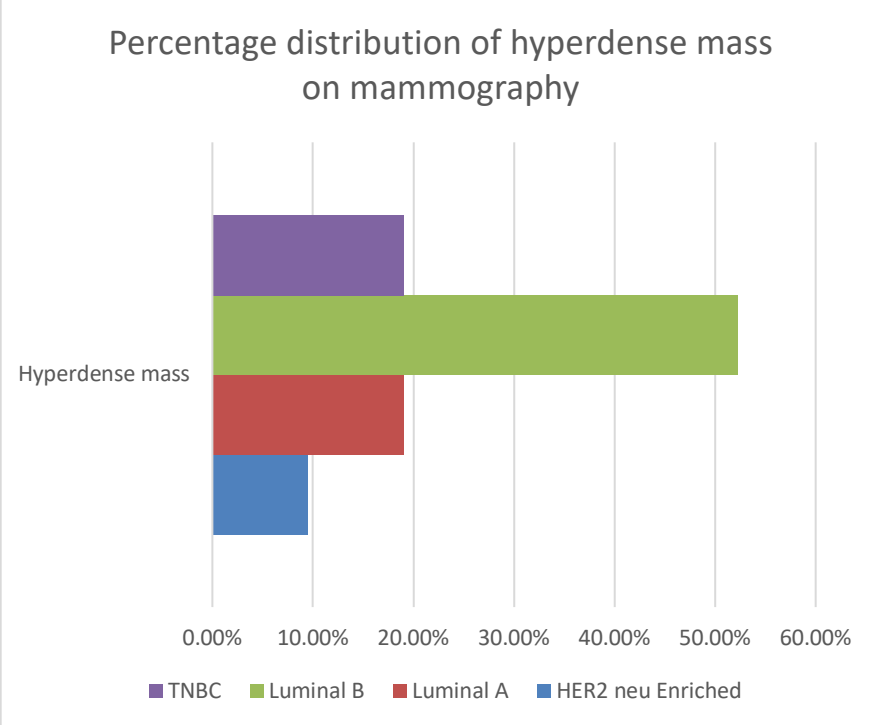


Figure 18: Percentage distribution of hyperdense mass in masses in different molecular subtypes in study subjects (n=142)

PRESENCE OF CALCIFICATIONS

Of our 154 patients, 72 (46.8%) patients had calcifications in the mass lesion detected on mammography.

Table 12: Types of suspicious calcifications in different molecular subtypes in study subjects (n=154)

		HER2 Neu enriched (n-43)	Luminal A (n-16)	Luminal B (n-64)	TNBC (n-31)	Total (n-154)
	Suspicious calcification	22 (51.2%)	7 (43.8%)	34 (53.1%)	9 (29%)	72 (46.8%)
Type of calci-fication	**Amorphous**	5 (11.6%)	1 (6.3%)	9 (14.1%)	0 (0.0%)	15 (9.7%)
	Coarse heterogenous	9 (20.9%)	6 (37.5%)	20 (31.3%)	6 (19.4%)	41 (26.6%)
	Fine pleomorphic	14 (32.6%)	3 (18.8%)	12 (18.8%)	6 (19.4%)	35 (22.7%)
	Fine linear	9 (20.9%)	1 (6.3%)	11 (17.2%)	4 (12.9%)	25 (16.2%)
	Fine linear branching	2 (4.7%)	0 (0.0%)	3 (4.7%)	0 (0.0%)	5 (3.2%)

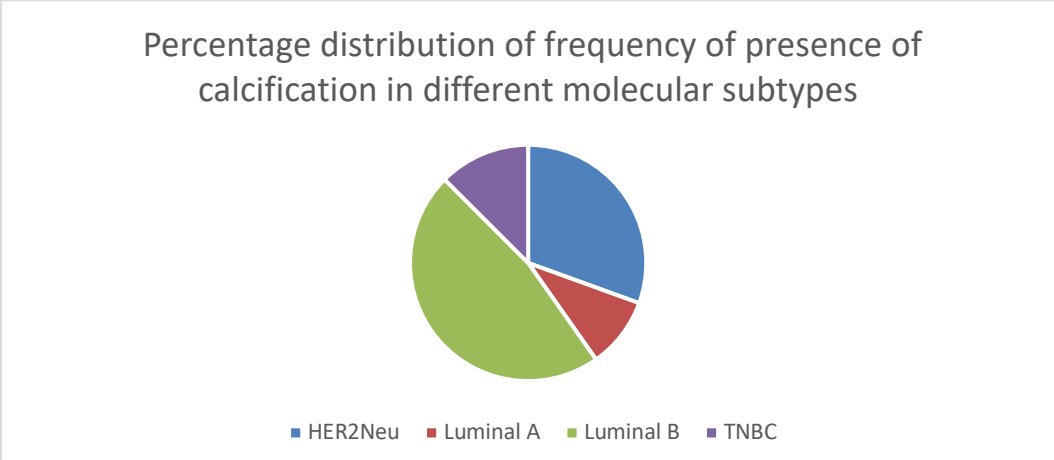


Figure 19: Percentage distribution of suspicious calcification in masses in different molecular subtypes in study subjects (n=154)

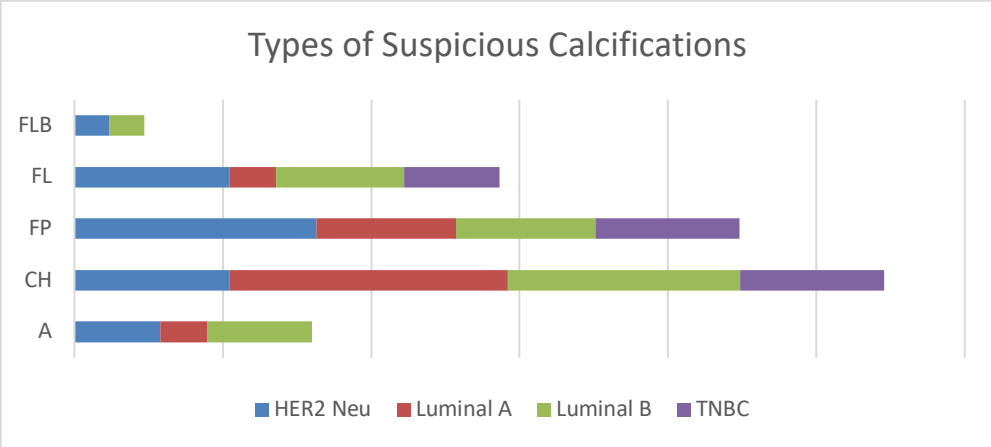


Figure 20: Percentage distribution of different types of suspicious calcification in masses in different molecular subtypes in study subjects (n=154)

About 53.1% of Luminal B patients had calcifications associated with the mass on mammography, of which coarse heterogenous calcifications were most common (31.3%) followed by fine pleomorphic type (18.8%).

HER2Neu subtype patients had maximum fine pleomorphic and fine linear calcification type (32.6%) followed by coarse heterogenous calcification (20.9%).

PRESENCE OF ARCHITECTURAL DISTORTION

Among 142 patients, the Luminal B subtype showed the maximum percentage of architectural distortion, followed by the HER2Neu enriched subtype.

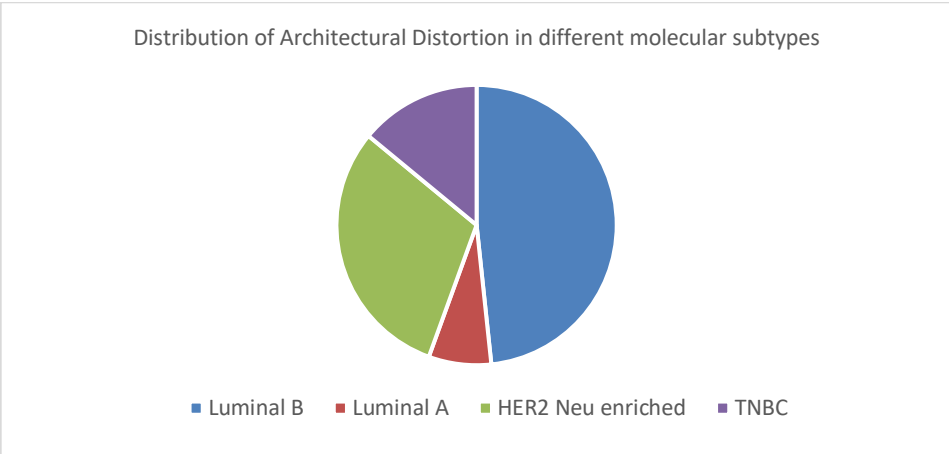


Figure 21: Percentage distribution of different types of architectural distortion

with masses in different molecular subtype (n=142).

ULTRASOUND FEATURES OF BREAST MASSES

Of our 154 patients, four patients had no appreciable mass in sonographic examination- out of which 3 were HER2 neu subtype and one patient was Luminal A subtype. Rest of the masses were analyzed statistically and the data is mentioned below.

SHAPE OF MASSES:

All patients with the Luminal A subtype presented with irregularly shaped lesions, while the presence of predominantly round or oval shapes was a feature of the TNBC subtype.

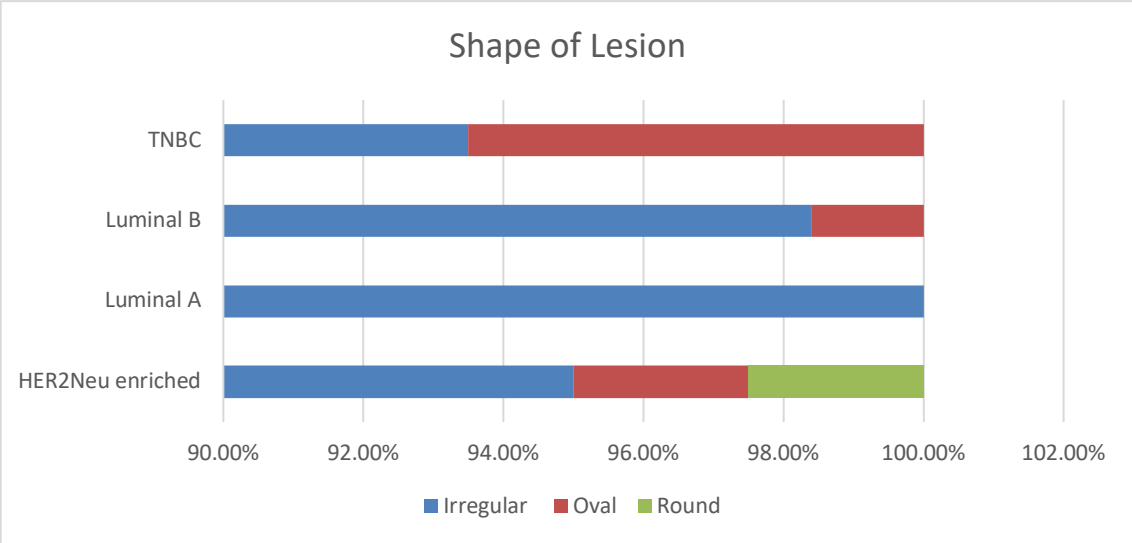


Figure 22: Percentage distribution of different types of shapes of masses in different molecular subtypes (n=150).

MARGINS ON ULTRASOUND:

All masses were evaluated for presence of uncircumscribed margins.

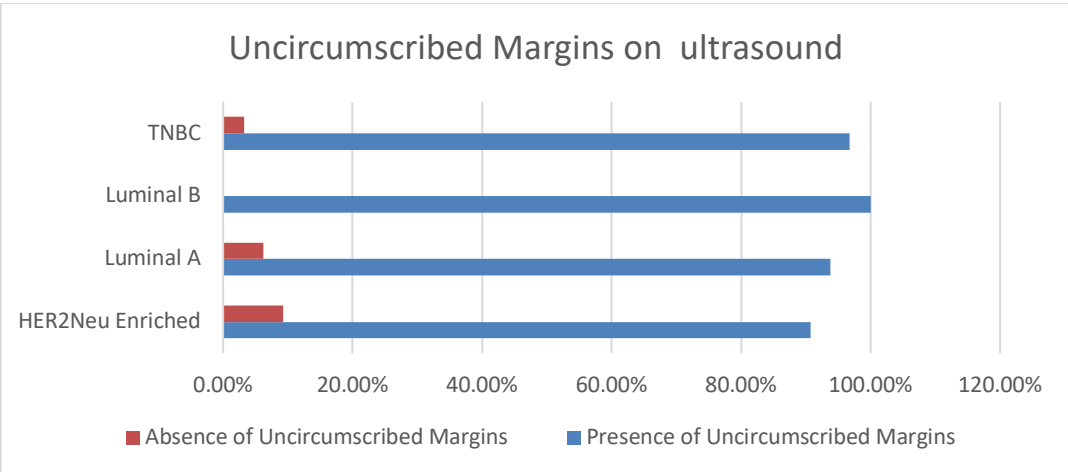


Figure23: Percentage distribution of presence of uncircumscribed margins in masses in different molecular subtypes (n=150).

ECHOPATTERN ON ULTRASOUND:

Of all the masses evaluated, 80.6% were hypoechoic masses on ultrasound examination. The rest, 29%, were heterogenous masses and solid cystic masses- the maximum of Luminal B masses were heterogenous, and among solid cystic lesions, the TNBC subtype showed solid cystic masses.

Table 13: Echo pattern of masses in different molecular subtype on histopathological in study subjects (n=150). **Four of our patients (3 HER2Neu subtype and 1 Luminal A subtype) showed no mass on ultrasonography and were excluded from n number in this table.*

Echo pattern	**HER2 Neu (n=40)**	**Luminal A (n=15)**	**Luminal B (n=64)**	**TNBC (n=31)**	**Total (n=150)**
Heterogenous echo pattern	0(0.0%)	0(0.0%)	19(29.7%)	1(3.2%)	20(13.3%)
Hypoechoic	39(97.5%)	12(80%)	44(68.8%)	26(83.9%)	121(80.6%)
Solid cystic pattern	1(2.5%)	3(20%)	1(1.6%)	4(12.9%)	9(6%)

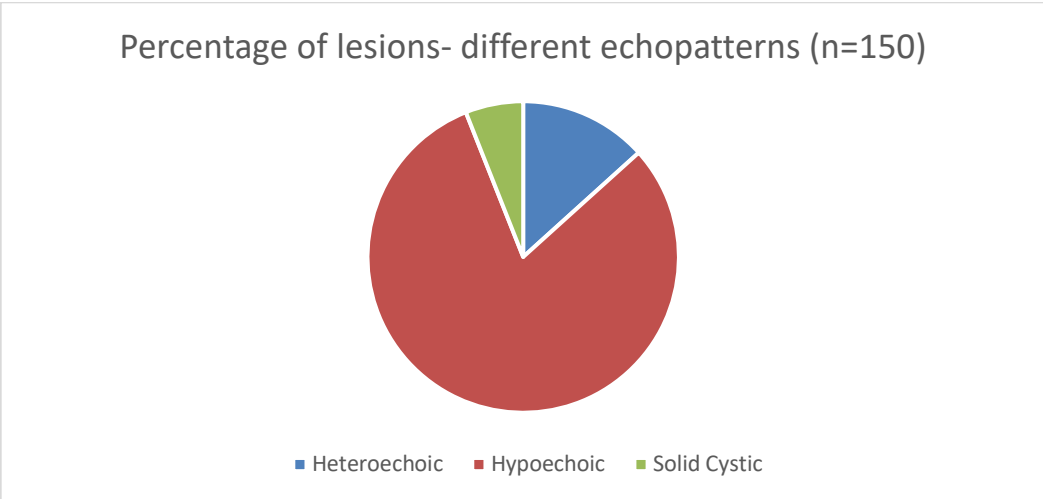


Figure 24: Percentage distribution of type of echo patterns in masses in different molecular subtype (n=150).

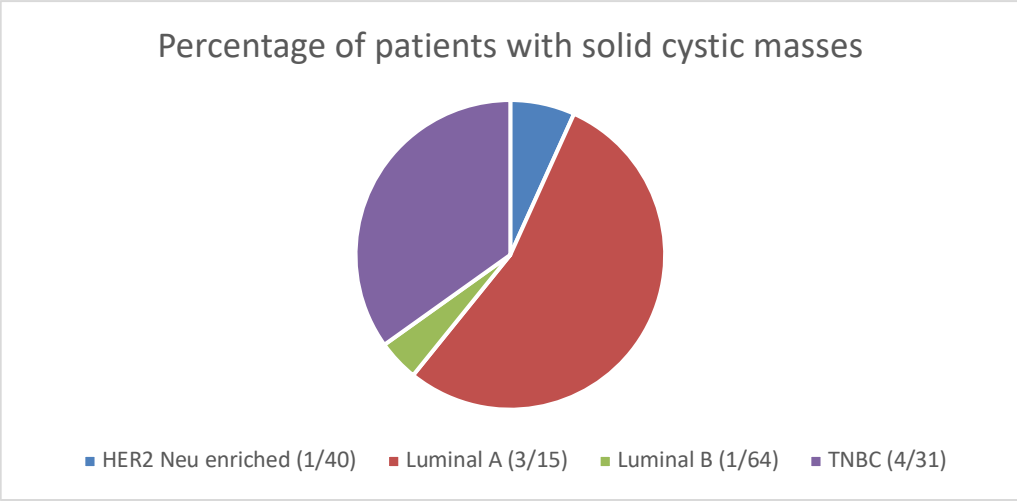


Figure 25: Percentage distribution of type of solid cystic echo pattern in masses in different molecular subtype (n=150).

POSTERIOR FEATURES OF BREAST MASSES ON ULTRASOUND:

Patients were evaluated for posterior acoustic features and three sub-groups were formed – masses with no posterior acoustic enhancement, masses with posterior acoustic shadowing and masses with posterior acoustic enhancement.

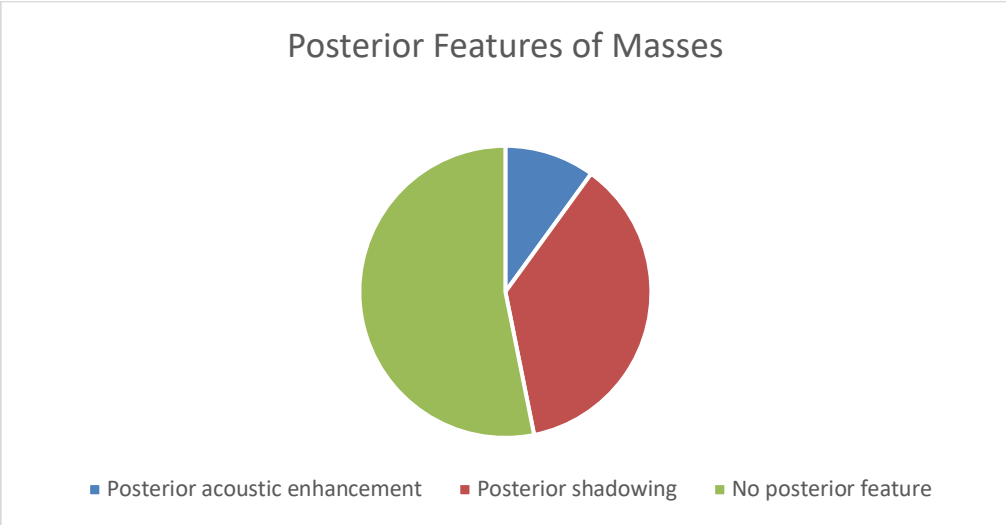


Figure 26: Percentage distribution of type of posterior features in masses (n=150).

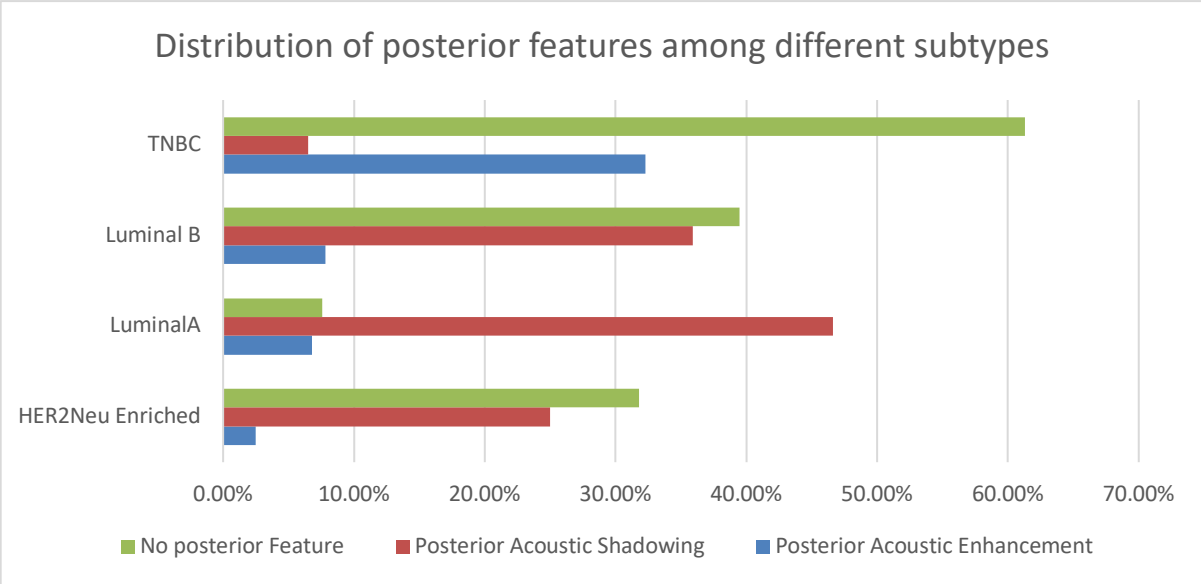


Figure27: Percentage distribution of type of posterior features in masses in different molecular subtype (n=150).

32.3% of TNBC subtype masses showed posterior acoustic enhancement. Posterior acoustic shadowing was found in only 6.5% TNBC patients. Rest of TNBC subtype showed no posterior features.

35.90% of Luminal B subtype masses showed posterior acoustic shadowing.

NON-MASS FEATURES- EVALUATION ON SONOGRAPHY:

Table 14: Associated non mass features of masses in different molecular subtypes in study subjects (n=154).

ASSOCIATED FEATURES	HER2 Neu (n=43)	Luminal A (n=16)	Luminal B (n=64)	TNBC (n=31)	Total (n=154)
Duct dilatation	9(20.9%)	4(25.0%)	10(15.6%)	5(16.1%)	28(18.2%)
Skin changes	6(14.0%)	3(18.8%)	11(17.2%)	7(22.6%)	27(17.5%)
Edema	5(11.6%)	0(0.0%)	8(12.5%)	3(9.7%)	16(10.4%)

Vascularity	43 (100.0%)	16 (100.0%)	64 (100.0%)	31 (100.0%)	154 (100.0%)

PRESENCE OF DILATED DUCTS WITH MASSES:

28 out of 154 of our patients (18.2%) had ductal dilatation in breast ultrasound. Frequency distribution of the cases has been shown below with various subtypes.

Table 15: Percentage of patients with ductal dilatation in different molecular subtypes (n=154).

	HER2 Neu (n=43)	**Luminal A (n=16)**	**Luminal B (n=64)**	**TNBC (n=31)**	**Total (n=154)**
Ductal Dilatation with/without mass on sono-mammography	9(20.9%)	4(25.0%)	10(15.6%)	5(16.1%)	28(18.2%)

Isolated ductal dilatation was not found in these subtypes in our patient population. 4 patients presented with isolated ductal dilatation of which 3 were HER2neu subtype and 1 was Luminal A subtype.

ASSOCIATION OF AXILLARY LYMPHADENOPATHY WITH MASSES

Presence of axillary lymphadenopathy was associated with all Molecular subtypes with varying frequencies.

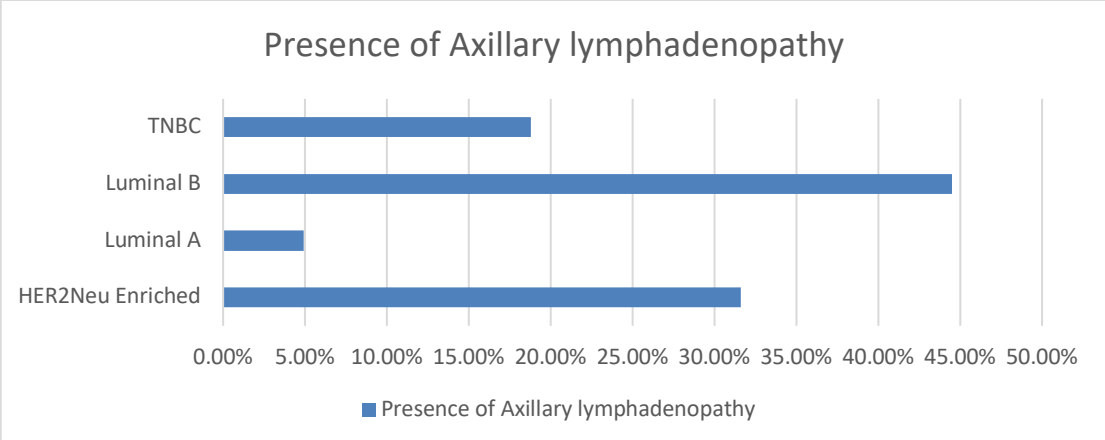


Figure 28: Presence of axillary lymphadenopathy was associated with all Molecular subtypes with varying frequencies.

Luminal B subtype had the maximum percentage of axillary lymphadenopathy with raised cortical thickness and effaced fatty hilum while most patients with Luminal A subtype did not show axillary lymphadenopathy.
Of various subtypes, cases with axillary lymphadenopathy were maximum with Nottingham Grade 2. Nottingham grade 1 patients had lesser association with axillary lymphadenopathy.

Table 16: Correlation of axillary node with Nottingham grade on histopathological in study subjects (n=154)

	Grade I (n=27)	**Grade II (n=90)**	**Grade III (n=37)**
N	15 (55.6%)	28 (34.5%)	7(18.9%)
Y	12 (44.4%)	59 (65.5%)	30(81.1%)

Based on the data collected, we tried to determine p values and analyze statistical relevance of our findings. The following are the details obtained.

Table 17: Mammography finding in molecular subtypes in study subjects (n=154)

	Her2 neu (n=43)	**Non HER2 neu (n=111)**	**P value**	**Luminal-A +ve (n=14)**	**Luminal A -ve (n=138)**	**P value**
Shape-Oval	1(2.75%)	2(1.8%)	1.0	0(0.0%)	3(2.1%)	1.0
Shape-Round	1(2.75%)	3(2.7%)	1.0	0(0.0%)	4(2.8%)	1.0
Shape-Irregular	35(81.3%)	100(90%)	1.0	14(100%)	121(87.6%)	1.0
Margin-Indistinct	1(2.7%)	5(4.5%)	1.0	0(0.0%)	6(4.3%)	1.0

Margin-Micro lobulated	25(67.5%)	67(60.3%)	0.80	6(42.8%)	86(62.3%)	0.06
Margin-Spiculated	8(21.6%)	28(25.2%)	0.38	8(57.1%)	28(20.2%)	***<0.01***
Hyperdense lesion	2(5.4%)	19(17.1%)	0.06	4(29.7%)	17(12.3%)	0.22
Suspicious calcification	22(51.2%)	50(45%)	0.21	7(43.8%)	65(47.1%)	0.95
Nipple retraction	4(9.3%)	28(25.2%)	0.06	3(18.8%)	29(21.0%)	1.0
Skin thickening	8(18.6%)	39(35.1%)	0.08	3(18.8%)	44(31.8%)	0.48

Table 18: Ultrasound finding in different molecular subtype in study subjects (n=150)

	Her2 neu (n=40)	**Her 2 neu negative (n=110)**	**P value**	**Luminal-A (n=15)**	**Luminal A -ve (n=140)**	**P value**
Presence of uncircumscribed margin	39(90.7%)	107(97.3%)	1.0	15(93.8%)	131(93.6%)	1.0
Micro lobulated Margin	32 (80%)	78(70.9%)	0.30	9(60.0%)	101(72.1%)	***<0.01***
Hypoechoic echo pattern	39(97.5%)	82(74.5%)	***<0.01***	12(80%)	109(77.9%)	***<0.001***
Posterior feature-Enhancement	1(2.5%)	16(14.5%)	***0.04***	1(6.8%)	16(11.4%)	0.69
Posterior feature-No	29(72.5%)	62(56.4%)	0.07	7(46.6%)	84(60.0%)	***0.01***
Posterior feature-Shadowing	10(25%)	32(29.1%)	0.62	7(46.6%)	35(25.0%)	0.25

Associated features -Duct dilatation	9(20.9%)	19(17.3%)	0.46	4(25.0%)	24(17.1%)	0.74
Axillary adenopathy	32(74.4%)	69(62.1%)	0.31	5(31.3%)	96(69.5%)	***<0.01***

The study population was divided on the basis of HER2 Neu enriched vs non-HER2 Neu enriched. Important imaging features on mammography were evaluated. Most of HER2 Neu enriched subtypes had irregular shape (81.3%), micro lobulated margins (67.5%) and presence of suspicious calcification (51.2%). However, this could not reach statistical significance. On sonography, the presence of hypoechoic mass (97.5%, p-value- <0.01) and the presence of posterior acoustic enhancement (2.5%, p value- 0.04) and irregular shape (90.7%, p-value -1.0).

Similarly, the study population was divided on the basis of Luminal A vs Non-Luminal A and an evaluation of important imaging features on mammography was done. Most of Luminal A subtype patients had spiculated margin (57.0%, p value - <0.01) on mammography. On sonography, presence of hypoechoic mass (80%, p value- <0.001) with no posterior acoustic feature (46.6%, p value- 0,01) and absence of associated axillary lymphadenopathy (p value- <0.01) was noted.

Table 19: Mammography finding in different molecular subtype in study subjects (n=154)

	Luminal-B (n=64)	**Luminal-B -ve (n=90)**	**p value**	**TNBC (n=31)**	**TNBC -ve (n=123)**	**P value**
Shape-Oval	0(0.0%)	3(3.3%)	0.26	2(6.6%)	1(0.8%)	0.10
Shape-Round	2(3.3%)	2(22.2%)	1.0	1(3.3%)	3(2.4%)	1.0
Shape- Irregular	59(92.1%)	76(84.4%)	0.698	27 (87.1%)	108 (87.8%)	0.163
Margin-Indistinct	5(8.1%)	1(1.11%)	0.08	0(0.0%)	6(4.8%)	0.60

Margin-Micro lobulated	36(59%)	56(62.2%)	0.45	25(83.3%)	67(54.4%)	***<0.01***
Margin-Spiculated	18(29.5%)	18(20%)	0.24	2(6.6%)	34(27.6)	***0.01***
Hyperdense lesion	11(18.1%)	10(11.1%)	0.34	4(13.3%)	17(13.8%)	1.0
Suspicious calcification	34(53.1%)	38(42.2%)	0.29	9(29.0%)	63(51.2%)	***0.01***
Nipple retraction	22(34.4%)	10(11.1%)	***0.001***	3(9.7%)	29(23.5%)	0.08
Skin thickening	28(43.8%)	19(21.1%)	***<0.01***	8(25.8%)	39(31.7%)	0.51

Table20: Ultrasound finding in different molecular subtype in study subjects (n=154)

	Luminal-B (n=64)	**Luminal B negative (n=86)**	**P value**	**TNBC (n=31)**	**TNBC -ve (n =119)**	**P value**
Presence of uncircumscribed margin	62(96.9%)	84(97.7%)	1.0	30(96.8%)	116(97.5%)	1.0
Micro lobulated Margin	42(65.6%)	68(79.1%)	0.06	27(87.1%)	83(69.7%)	0.05
Hypoechoic echo pattern	44(68.8%)	*77(89.5%)*	***0.001***	26(83.9%)	95(79.8%)	0.61
Posterior feature-Enhancement	5(7.8%)	12(14.0%)	0.30	10(32.3%)	7(5.9%)	**<0.001**
Posterior feature-No	36(56.3%)	55(64.0%)	0.33	19(61.3%)	72(60.5%)	1.0
Posterior feature-Shadowing	23(35.9%)	19(22.1%)	0.06	2(6.5%)	40(33.6%)	**<0.001**

Associated features -Duct dilatation	10(15.6%)	18(20.9%)	0.40	5(16.1%)	23 (19.3%)	0.8
Axillary adenopathy	45(70.3%)	56(62.2%)	0.25	19(61.3%)	82(66.6%)	0.76

The study population was divided on the basis of Luminal B vs non-Luminal B patients. Important imaging features on mammography were evaluated. Most of Luminal B patients' subtypes had irregular shape (92,1%), micro lobulated margins (59%) and presence of suspicious calcification (53.1%). However, this could not reach statistical significance. On sonography, the presence of hypoechoic mass (68.8%, p-value- <0.001) with micro lobulated margins (65.6% p value- 0.06), and the presence of posterior acoustic shadowing (35.9%, p value-0.06) or no posterior feature (56.3%, p-value -0.33) were noted.

Similarly, the study population was divided on the basis of TNBC vs non-TNBC and an evaluation of important imaging features on mammography was done. Most of TNBC subtype patients had micro lobulated margins (83.3%, p value <0.01) with absence of spiculated margin (6.6%, p value - <0.01) and absence of suspicious calcification (29%, p value- 0.01) on mammography. On sonography, presence of posterior acoustic enhancement (32.5%, p value- <0.001) with absence of posterior acoustic shadowing (33.6%, p value- <0.01) was noted.

DISCUSSION:

Infiltrating Ductal carcinoma (IDC-NOS) can present with different features on mammography and sonography. Better knowledge of these features and their histopathological correlations may help predict tumor biological behavior.

A total of 154 patients were included in the study, with the age of patients ranging from 34-86 years age. In comparison to the Western population, the greatest role of mammography is observed in the group of women aged 50–69 years. A global increase in the incidence of breast cancer is observed in all age groups and is highest in women under 50 years of age (40). In Indian population, a younger age of breast cancer incidence is noted. Studies suggest that the disease peaks at 40-50 years in Indian women (41). In our study, the average age of presentation was 52 ±11 years.
Many of these cancers are HER2 positive and ER/PR negative, or HER2/ER/PR all three negative, and have a poor prognosis (41). Younger patients tend to be triple negative, which is a significant poor prognostic factor.40 Prevalence of TNBC in India is considerably higher compared with that seen in Western populations as suggested by various meta-analysis studies. (42) In our study, of all the molecular subtypes, Triple Negative breast carcinoma (TNBC) shows the earliest mean age of presentation (49.10±10.34), while the Luminal A subtype showed the latest mean age of presentation (55.63±14.03).
Women menstruating at age <12 years have more chances of developing breast carcinoma than women with >12 years age of menarche (41). In our study, the average age of menarche was 13.45 (SD-1.48) years. Of all the molecular subtypes, the HER2neu enriched subtype has the earliest age of menarche, while the Luminal A subtype showed the latest mean age of menarche. However, this could not be substantiated statistically (p- value 0.138).

Having extremely dense breast tissue (BI-RADS density D) resulted in a 2.11-fold (95% CI 1.84–2.42) increased breast cancer risk compared to having scattered dense breast tissue (BI-RADS density B) (43). **Park *et al*. also** demonstrated that there is a significant association between HER2-positive tumors and higher breast density.(6) In our study, we also observed a positive relationship between heterogeneous dense

breasts and HER2-positive status. However, it did not demonstrate statistical significance (p- value- 0.124).

TNBC is a high-grade tumor histologically, and many studies have shown a worse prognosis in patients with the TNBC subtype. On comparison of clinical profile, TNBC tumors were significantly large, with the majority of patients presenting as locally advanced breast cancer (83%). (44) In our study, most of the masses were in the range of size 2.1-5 cm (65.4%).16.9% of masses had a size range of 1.1-2 cm. Only 17.5% of patients had masses of size more than 5 cm in maximum dimension. TNBC subtype patients had large masses on presentation.
In our study, luminal-B and triple-negative subtypes were more likely to have a higher Nottingham grade (67.5% of Luminal B and TNBC combined had Nottingham grade 3). The Luminal-A subtype correlated with lower histological grades (100% Luminal A subtype had grade 1 (62.5%) and grade 2 (37.5%) Nottingham grade).

Studies have shown that triple-negative subtypes have more oval or round shapes and more circumscribed margins; however, micro-lobulated and obscured margins were also frequent. (40). Other studies however have also shown that the majority of TNBCs (48%–85%) manifest as irregularly shaped masses without calcifications. Margins most commonly are ill defined or indistinct (45%–46% of cases) or spiculated (15%–21% of cases) (38). In our study, 87.5% triple-negative subtypes had irregular shape with micro lobulated margins (P value- <0.01) and few had oval shapes on mammography. Absence of spiculated margin and absence of suspicious calcification in TNBC subtype showed high statistical significance (P value- <0.001) in our study. On Ultrasonographic examination, the presence of posterior acoustic enhancement and absence of posterior acoustic shadowing were also noted, which showed high statistical significance (P value- <0.001) in our study.

Jiang L *et al* found that spiculated margins have a significant association with positive hormone receptor status. Spiculated masses also tend to have HER2 negativity and a lower Ki-67 label index in comparison to non-spiculated masses.(44) In our study, Luminal A had a significant correlation with spiculated margin on mammograms (57.1% and p value <0.01 in Luminal A) compared to 21.6% and p- value-0.38 in

HER2Neu positive patients.

Many studies have shown that the HER2neu-enriched subtype has a strong association with the presence of microcalcifications. In our study, 50% of HER2neu enriched patients had associated suspicious microcalcifications - HER2Neu subtype patients had a maximum of fine pleomorphic and fine liner type of calcification (32.6% each) followed by coarse heterogeneous calcification (20.9%). However, this could not reach to statistical significance (p-value- 0.21). HER2Neu subtypes in our study showed a positive correlation with hypoechoic echo pattern (p-value- <0.01) and posterior acoustic enhancement (p-value- 0.04). A large number of HER2Neu enriched patients in our study had no posterior acoustic feature (72.5%).

In our study, the Luminal B subtype showed a positive correlation with mammographic features of skin thickening and nipple retraction (p- value <0.01 for both). On mammography, about 20.3% of Luminal B subtype masses had indistinct margins with surrounding breast parenchyma, which appeared as one of the conspicuous features; however, it could not reach statistical significance (p-value- 0.08). On Ultrasonography, most Luminal B masses were hypoechoic in echo pattern (p value- 0.01). Although micro lobulated margins were noted in most of the Luminal B subtype (59%), this could not reach statistical significance. 18% of Luminal B subtype patients had a hyperdense mass on mammography. However, this could not reach statistical significance (p-value – 0.34). 18.8% of Luminal B subtype patients had an anti-parallel orientation of masses; however, this was not statistically significant (p-value- 0.55).

Brahami *et al* showed that masses with ER-positive/PR-positive status were associated with spiculated sonographic margins (39). In our study, the Luminal A subtype had a very strong correlation with the presence of spiculated margins (57.1%, p-value- <0.01). The Luminal A subtype was not associated with axillary lymphadenopathy, and this feature had a strong statistical significance (p-value- <0.01). A significant number of Luminal A subtype patients (35%) had posterior acoustic shadowing and 6.8% had posterior acoustic enhancement.

SUMMARY:

To summarize, this was a sono-mammography based prospective observational cross sectional study conducted on central India population in AIIMS Bhopal, in which an attempt to evaluate the imaging signatures of different molecular subtypes of infiltrating ductal carcinoma variety of breast cancer was made.

We found that triple-negative subtypes had irregular shape with micro lobulated margins with absence of spiculated margin and absence of suspicious calcification on mammography. On Ultrasonographic examination, presence of posterior acoustic enhancement and absence of posterior acoustic shadowing was found.

Luminal A molecular subtype had a significant correlation with spiculated margin on mammograms. On ultrasound examination, association with posterior acoustic shadowing and absence of associated with axillary lymphadenopathy was found.

HER2neu enriched patients had associated suspicious microcalcifications. HER2Neu subtype patients had maximum of fine pleomorphic type of calcification followed by coarse heterogenous calcification On sonography, HER2Neu subtype in our study showed positive correlation with hypoechoic echo pattern and posterior acoustic enhancement. A large number of HER2Neu enriched patients in our study had no posterior acoustic feature (72.5%).

Luminal B subtype showed on mammography had indistinct margins as one of the conspicuous feature. Associated features of skin thickening and nipple retraction were found. On Ultrasonography, most Luminal B masses were hypoechoic in echo pattern and had micro lobulated margins.

LIMITATIONS

Our study included patients who presented with mass/breast pain/ nipple discharge etc. and screening mammography was not done at our institute. This precluded the estimation of true incidence of various subtypes and their prevalence could only be calculated on basis of patient population who came to our institute. The study was conducted in a single institute.

Only those patients whose histopathology and IHC was available during our study period could be included in the study. This led to decrease in total sample size.

CONCLUSION:

The mammography and sonography features may reflect the biological behavior of various subtypes of breast cancer. These findings may be an excellent predictor for some subtypes like triple-negative breast cancer. ER, PR, HER2, and Ki-67 expression have prognostic and therapeutic values in breast cancer. Studying the range of these imaging characteristics may help in better understanding the prognosis, choosing a treatment strategy, and predicting response to treatment.

FUTURE SCOPE:

The diagnostic performance of breast imaging modalities may result in “imaging signatures” specific for molecular breast cancer subtypes. This may lead to improved and earlier detection of more aggressive breast cancer subtypes and may raise thresholds for recall and biopsy by identification of indolent disease. Identification of women with high risk of biopsy sampling error due to tumor heterogeneity may be segregated based on high index of suspicion and repeat targeted biopsy may be done. The relation between imaging features and cancer behavior may have prognostic relevance.

The application of Radiomics in the evaluation of breast carcinoma is evolving, and it is a translational field of research aiming to find associations between quantitative and qualitative information extracted from medical imaging to allow the creation of high-dimensional data with available clinical data. Radiomics offers great potentialities that can be explored in coming future.

FUTURE RECOMMENDATIONS:

Our study had a limited number of patients as many patients did not undergo histopathological examination, the histopathology of many patients was not available, and few patients could not get immunohistochemistry done. More stringent single sitting targeted biopsies with lesser drop-out rates may ensure better management for patients.

The operator dependence of ultrasound as an imaging modality is a challenge. Standardized institution-based protocol and regular training sessions may help decreasing inter-observer bias.

REPRESENTATIVE CASES:

Case 1: A 55-year-old female came with complaints of a lump in right breast since last 6 months. HISTOPATHOLOGICAL and IHC revealed HER2neu enriched.

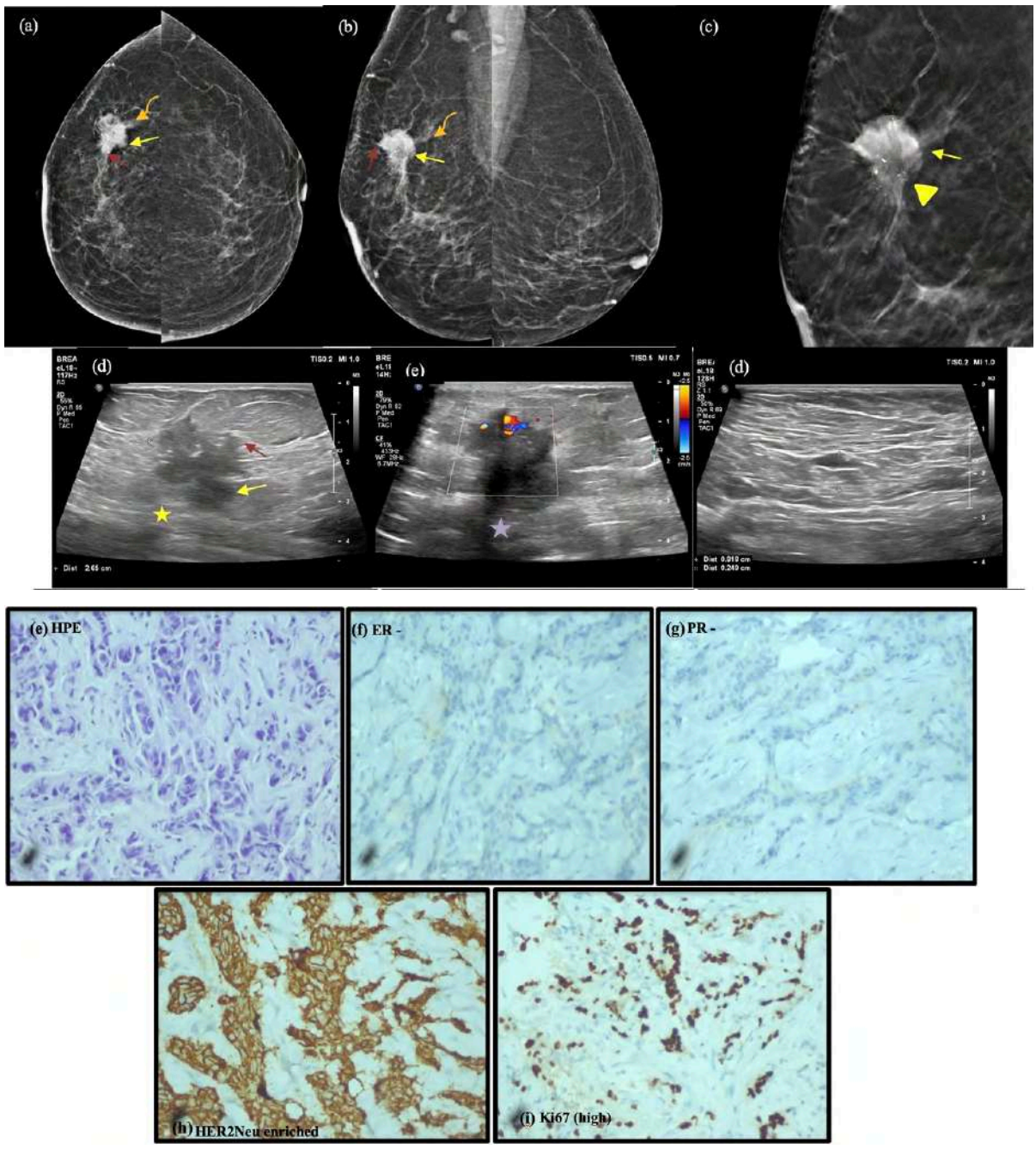


Figure29: Craniocaudal view (a), mediolateral view (b) of bilateral breasts and magnified image (c) of the mass of the right breast shows an irregularly shaped homogenously iso-dense mass in the central quadrant with micro-lobulated margins (yellow arrows) and few areas of speculations (red arrow). Surrounding breast parenchyma shows architectural distortion (curved ochre arrow). Few suspicious

microcalcifications of fine pleomorphic variety and coarse heterogenous variety are noted within the lesion. On Ultrasound correlation, an irregularly shaped hypoechoic mass in anti-parallel orientation with micro lobulated (yellow arrow) and spiculated (red arrow) margin is noted at 11-12 o'clock. It shows mixed posterior acoustic enhancement (yellow star) and posterior acoustic shadowing (purple star). A few small internal hyperechoic foci are noted. No e/o associated dilated ducts are pointed out. The bilateral axilla showed sub-centimetric lymph nodes with maintained fatty hilum. Histopathological and immunohistochemistry images (e-i) represent HER2 neu enriched status of mass.

Case 2: A 73-year-old female presented with complaints of a lump in her right breast for seven months. On histopathology and IHC- Luminal A subtype.

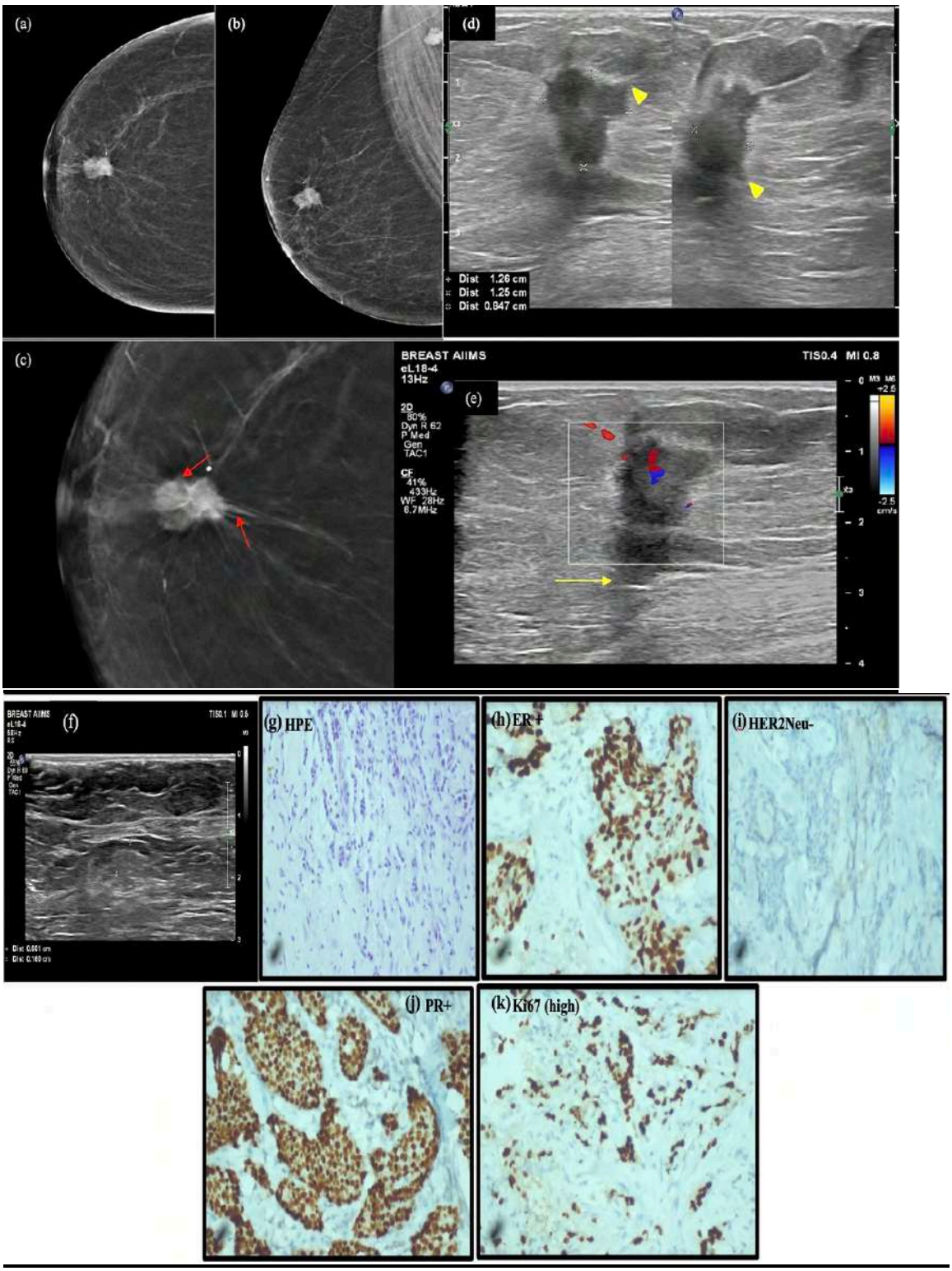


Figure 30: Craniocaudal (a), MLO (b) view and a tomosynthesis view focused on the region of interest (c) of the left breast shows a well-circumscribed irregularly shaped heterogeneously iso-dense mass in the central upper quadrant with spiculated margins. Surrounding breast parenchyma shows architectural distortion and trabecular thickening. No e/o any suspicious microcalcification is noted. On Ultrasound correlation- (d), (e) an irregularly shaped homogenously hypoechoic mass in anti-parallel orientation with spiculated margins (arrowheads) and raised internal vascularity in the retro mammary region. It shows posterior acoustic shadowing (yellow arrow mark). They surround breast parenchyma with mild architectural distortion. No e/o any internal hyperechoic foci/associated dilated ducts are noted. The right axilla showed few sub-centimetric lymph nodes with maintained fatty hilum. Images (g) to (k)- show histopathological and immunohistochemistry images showing Luminal A status.

Case 3: A 58-year-old female presented with complaints of a lump in her right breast with nipple retraction and nipple retraction for the last year. On histopathology and IHC, the Luminal B subtype was found.

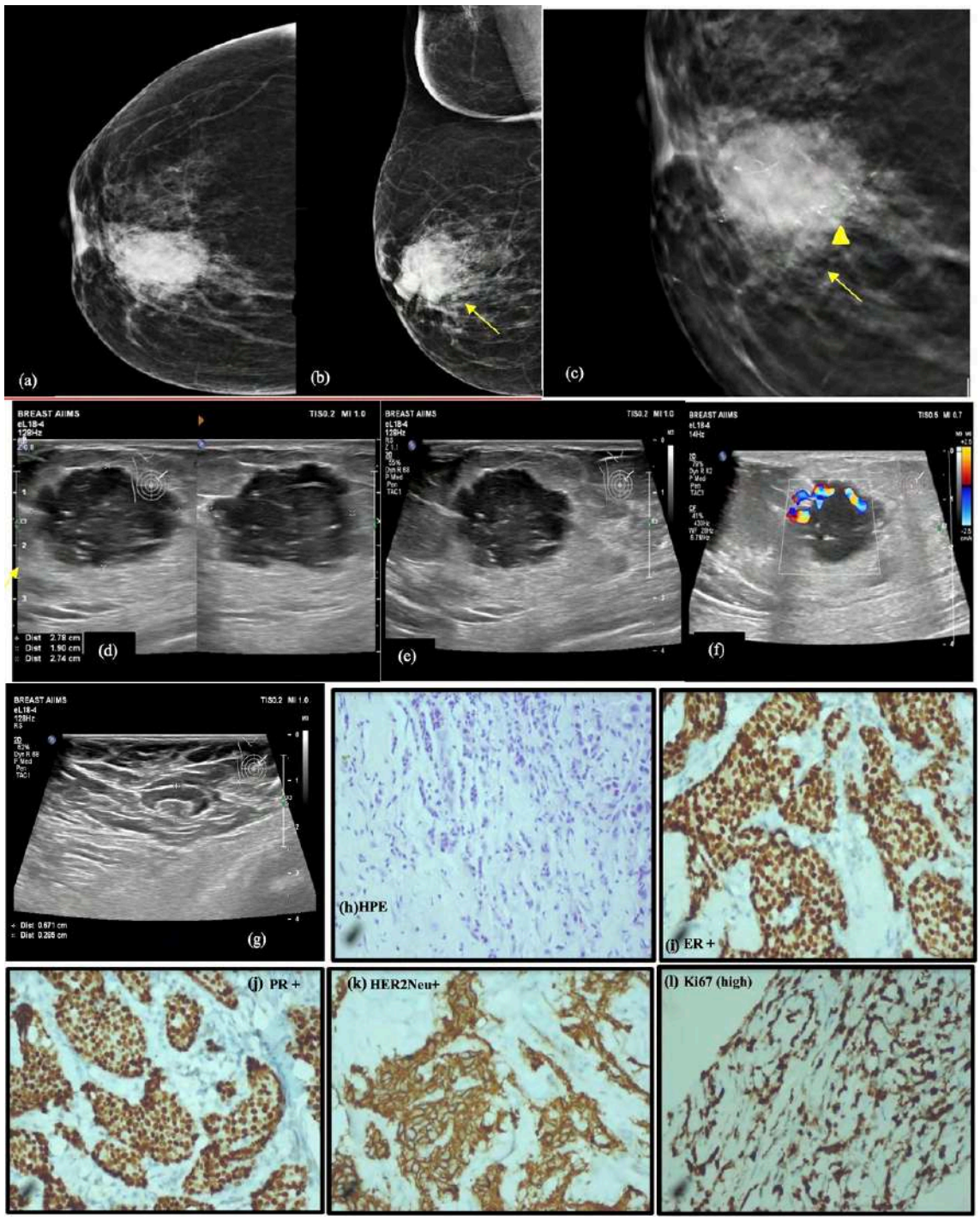
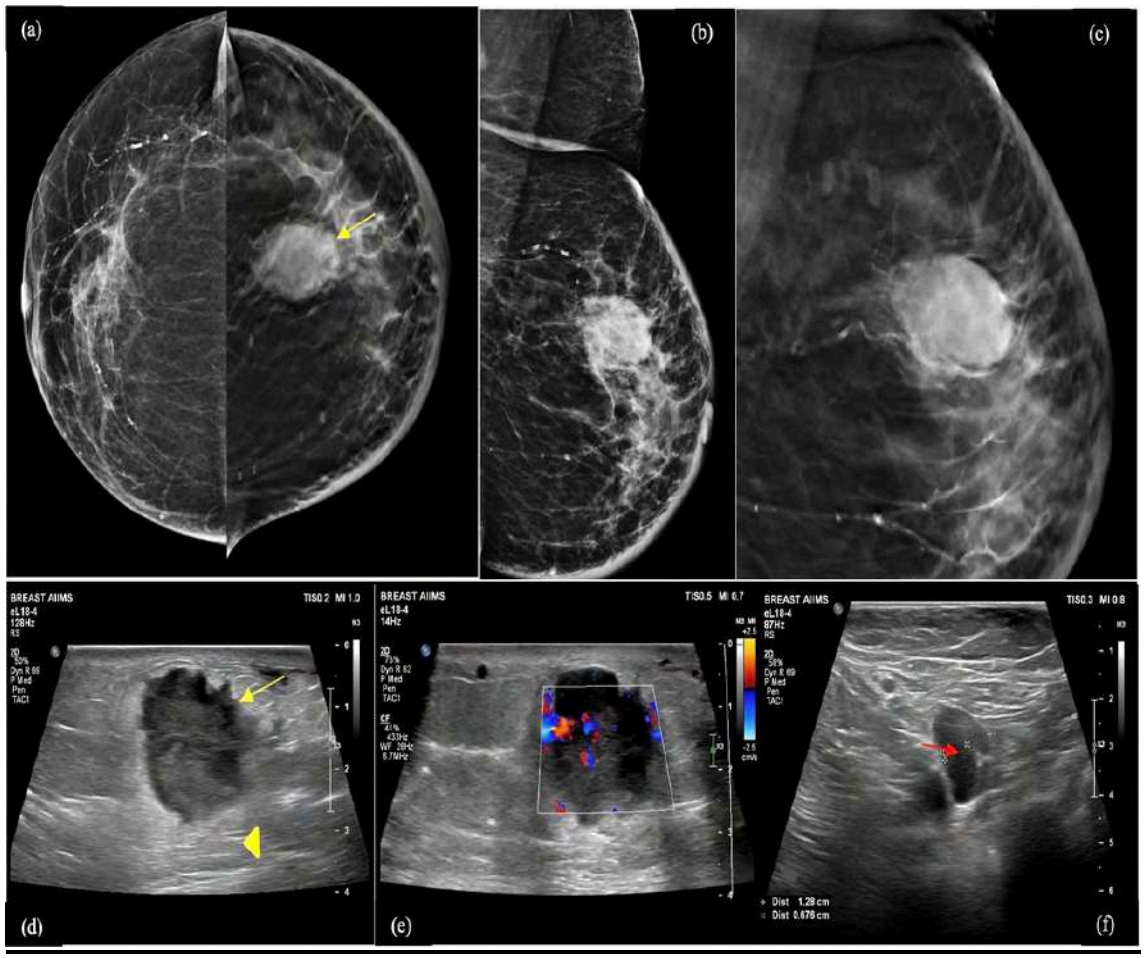


Figure31: Craniocaudal (a) and MLO (b) view & tomosynthesis view focused on the region of interest (c) of the left breast shows a relatively defined irregularly shaped heterogeneously iso-dense mass in the upper quadrant with more than three micro-lobulations and indistinct margin in some parts (represented by yellow arrow marks). A

few suspicious microcalcifications of fine pleomorphic variety are noted in the inferior aspect of the lesion (represented by a yellow arrowhead). On ultrasound correlation, an irregularly shaped hypoechoic mass is in parallel orientation with micro-lobulated margins, and internal vascularity is raised in the retro-areolar region. No apparent posterior features could be seen. The lesion shows indistinct planes with surrounding breast parenchyma in some places. Few internal hyperechoic foci are noted. No e/o any associated dilated ducts are noted. (g)- Subcentimetric lymph nodes with maintained fatty hilum are noted in the bilateral axilla. (f)- (l)- Histopathological and IHC images representing Luminal B positive subtype

Case 4: A 69-year-old female presented with complaints of a lump in the left breast showing a rapid increase in size in the last 15 days. On histopathology &IHC, molecular subtype TNBC was found.



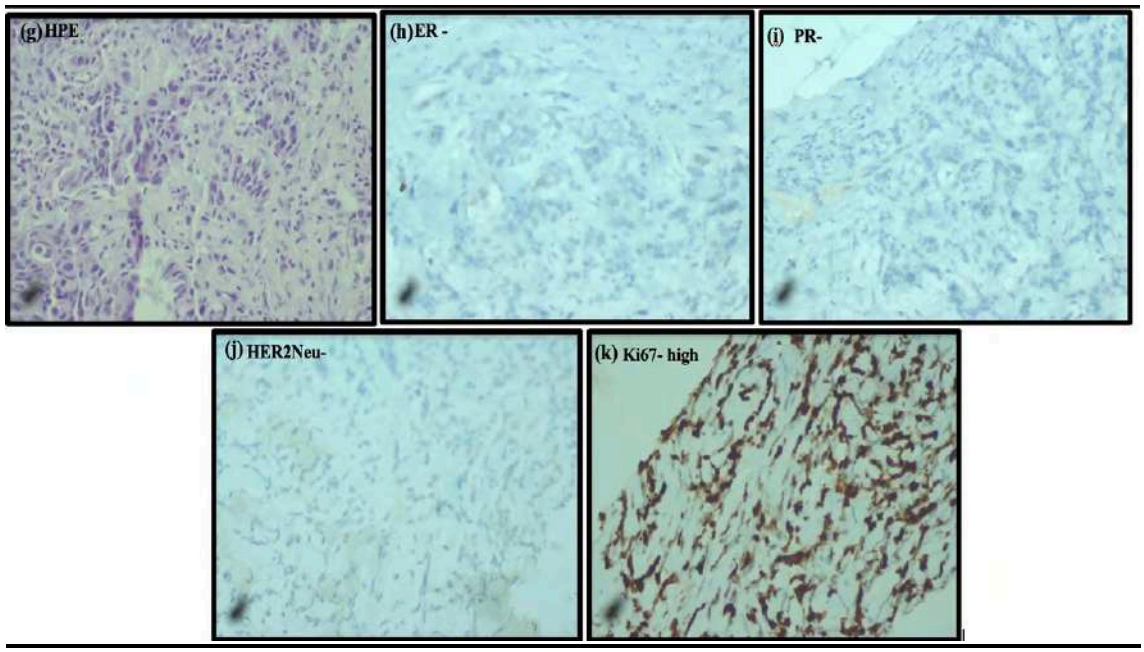


Figure 32: Craniocaudal (a) and MLO (b) view and (c) focused on the region of interest of the left breast shows a relatively well-circumscribed irregularly shaped homogenously iso-dense mass in the central upper quadrant with more than three micro-lobulations (represented by yellow arrow marks). Surrounding breast parenchyma shows mild architectural distortion and trabecular thickening. Mild skin thickening is also noted. No e/o suspicious microcalcification is indicated. On Ultrasound correlation, an irregularly shaped homogenously hypoechoic mass in anti-parallel orientation with micro-lobulated margins and raised internal vascularity at the 11-12 o'clock position. It shows posterior acoustic enhancement. Surrounding breast parenchyma showed edematous changes and mild skin thickening. No e/o any internal hyperechoic foci/associated dilated ducts are noted. The left axilla showed few enlarged lymph nodes with effaced fatty hilum and raised cortical thickness. (g)- (k)- Histopathological and IHC images representing the TNBC subtype

FEW OTHER CASES FROM DIFFERENT SUBTYPES

Case 1: A 58-year-old female came with c/o left breast nipple excoriation and bloody discharge. Histopathology and IHC revealed HER2Neu enriched molecular subtype with the following imaging findings:

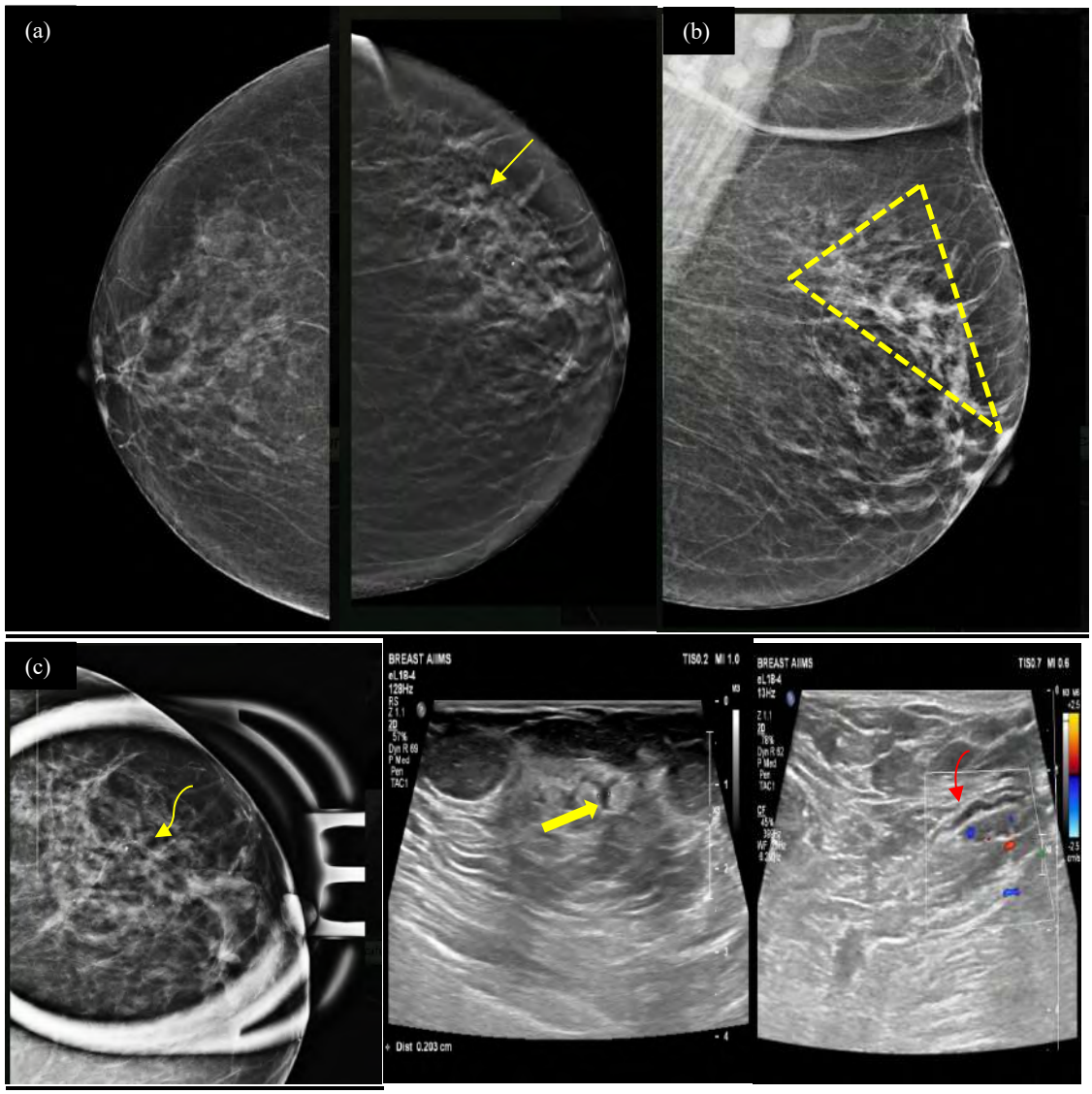
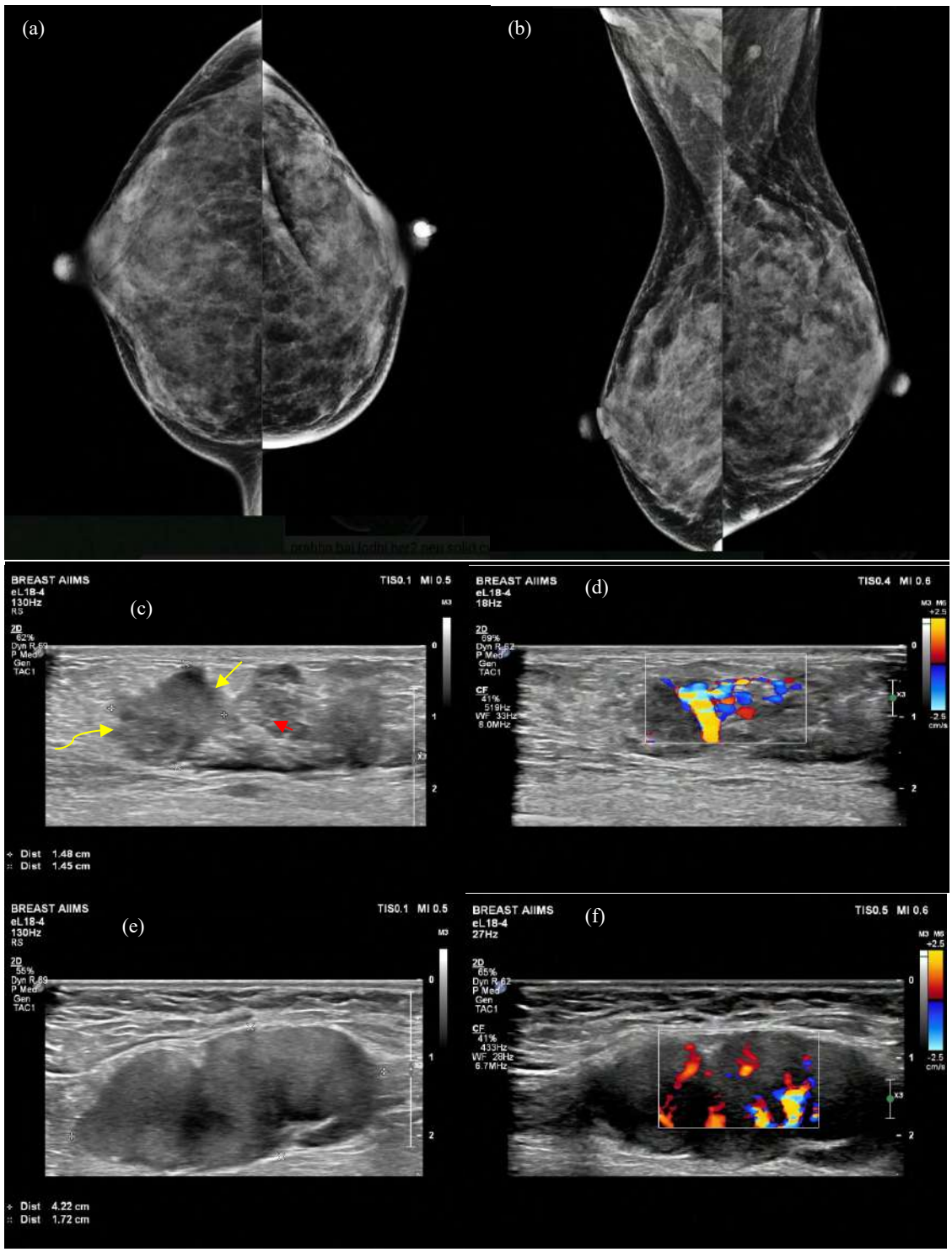


Figure 33- (a) Bilateral CC view and (b) left MLO view and (c) compression and magnification view of the left breast (type B) show multiple suspicious- fine pleomorphic, fine linear and few coarse heterogeneous microcalcifications in regional distribution (dashed yellow triangle) in upper outer and central quadrant with some of them extending till retro-areolar region. Focal asymmetry (yellow arrow) is also noted in the upper outer quadrant (in comparison to the contralateral breast). On Ultrasound correlation, multiple prominent ducts (maximum diameter ~ 2.4 mm) [yellow block arrow] are noted in the upper outer quadrant extending till the peri-areolar region, with some of them showing internal echoes and mildly thickened walls [red curved arrow], however no evidence of any raised vascularity or solid component noted.

Case 2: A 34-year-old female came with c/o left breast lump x 4 months with yellowish nipple discharge. Histopathology and IHC revealed HER2Neu enriched molecular subtype with the following imaging findings:



Figures34: (a) Bilateral CC view and (b) MLO view of bilateral breasts (type D) shows no mass or suspicious microcalcification. (c), (d) show ultrasound images of the left

breast, which revealed an irregularly shaped ill, defined heterogeneously hypoechoic lesion with multilobulated (yellow straight arrow) and spiculated margins (yellow curved arrow) with tiny echogenic foci (red arrow) and increased vascularity. (e) and (f) represent enlarged lymph nodes with eccentric fatty hilum, raised cortical thickness and internal vascularity (suspicious nodes).

Case 3: A 40-year-old female came with c/o right breast lump x 8 days with associated pain. Histopathology & IHC revealed HER2Neu enriched molecular subtype with the following imaging findings:

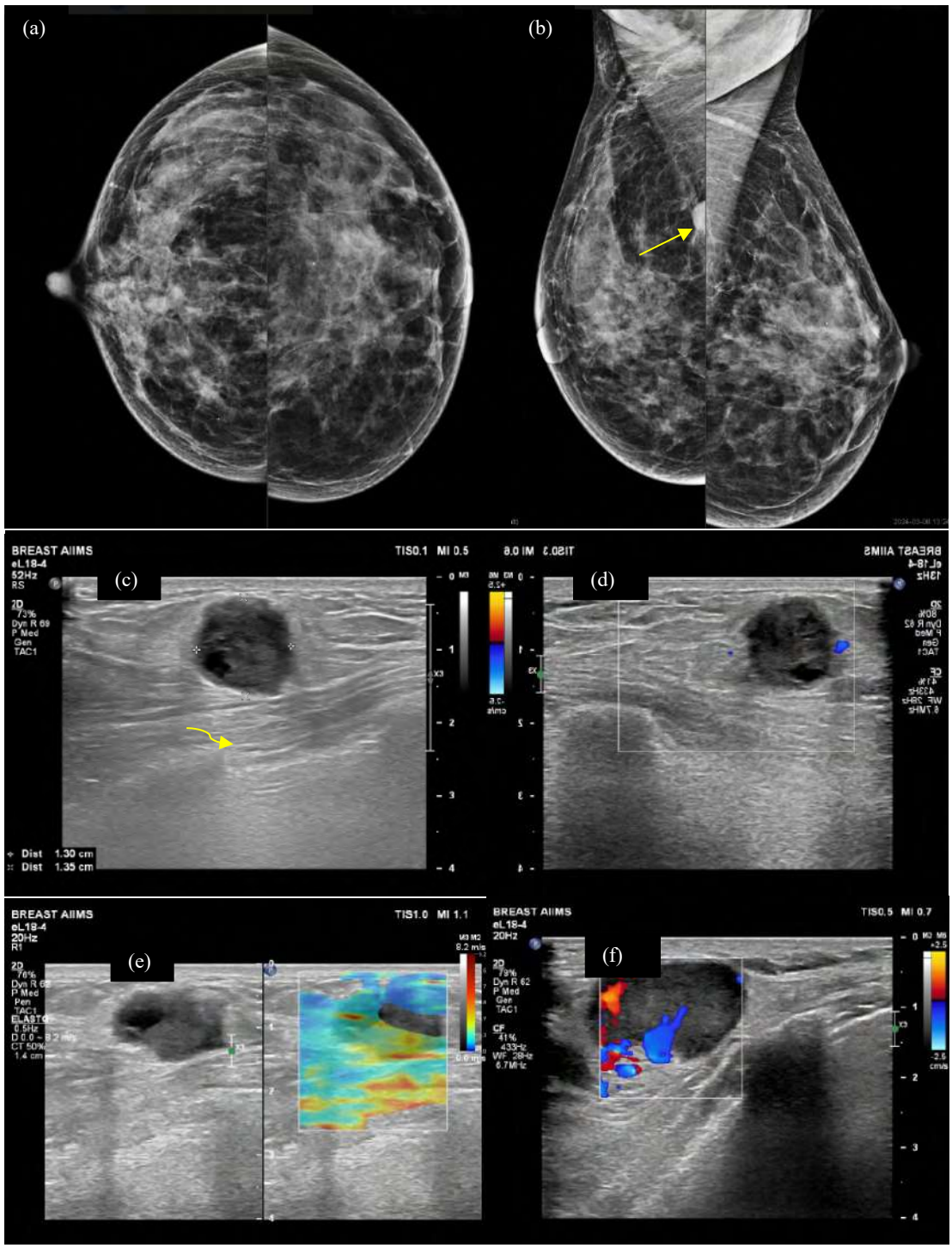


Figure35- (a) Bilateral CC view and (b) MLO view of bilateral breasts (type C) shows a partly visualized well-circumscribed round isodose mass near the chest wall in MLO

view (yellow arrow). (c), (d) show ultrasound images of the left breast which revealed a round heterogenous solid cystic lesion with a solid part with partly micro lobulated and partly angular margins showing internal vascularity and on elastography (e) shows a soft consistency. Posterior acoustic enhancement is noted (yellow curved arrow). (f) represents enlarged lymph nodes with eccentric fatty hilum, raised cortical thickness and internal vascularity (suspicious nodes)

Case 4: A 65-year-old female came with c/o left breast lump x 6 months. Histopathology & IHC revealed Luminal A molecular subtype with the following imaging findings:

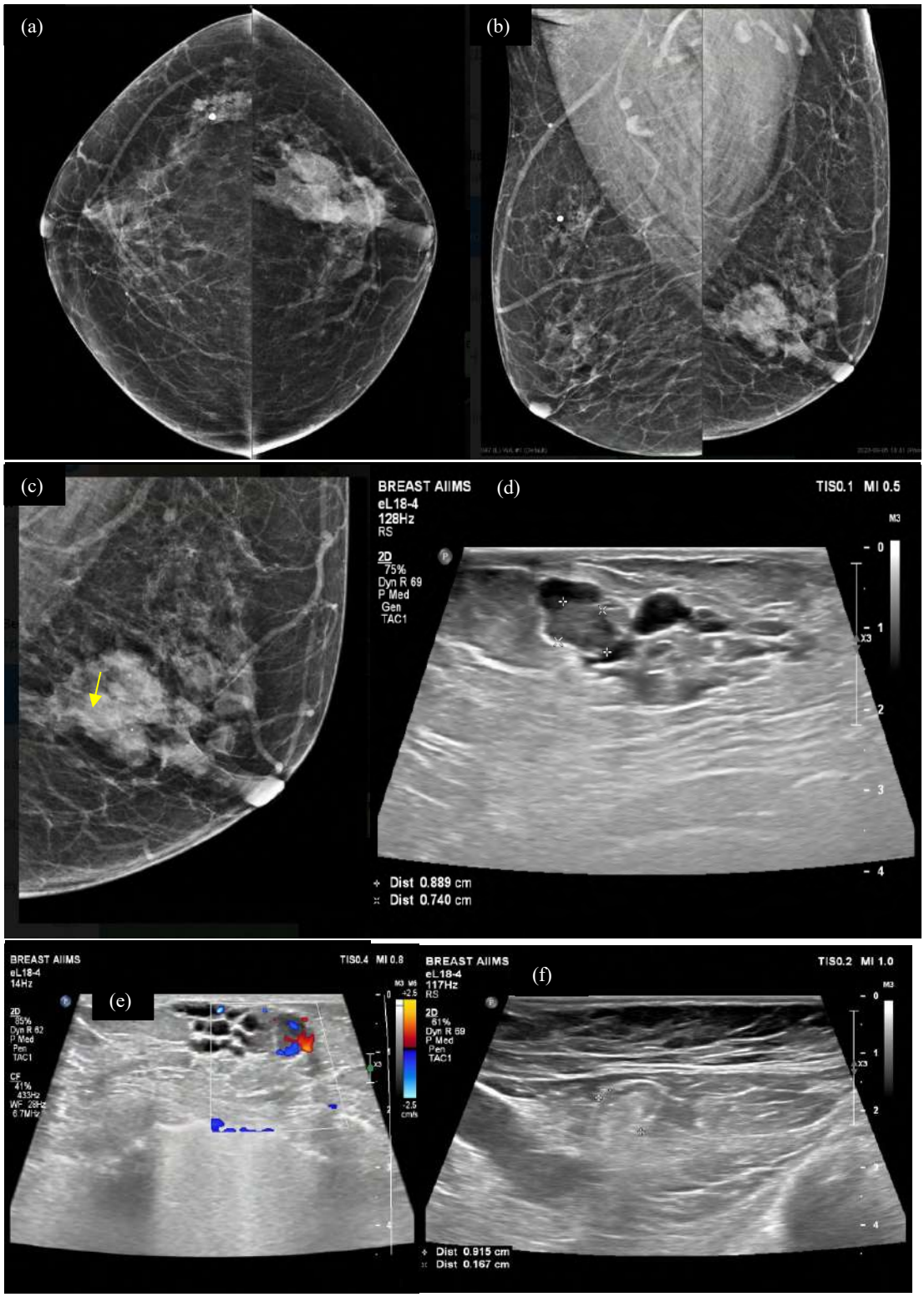
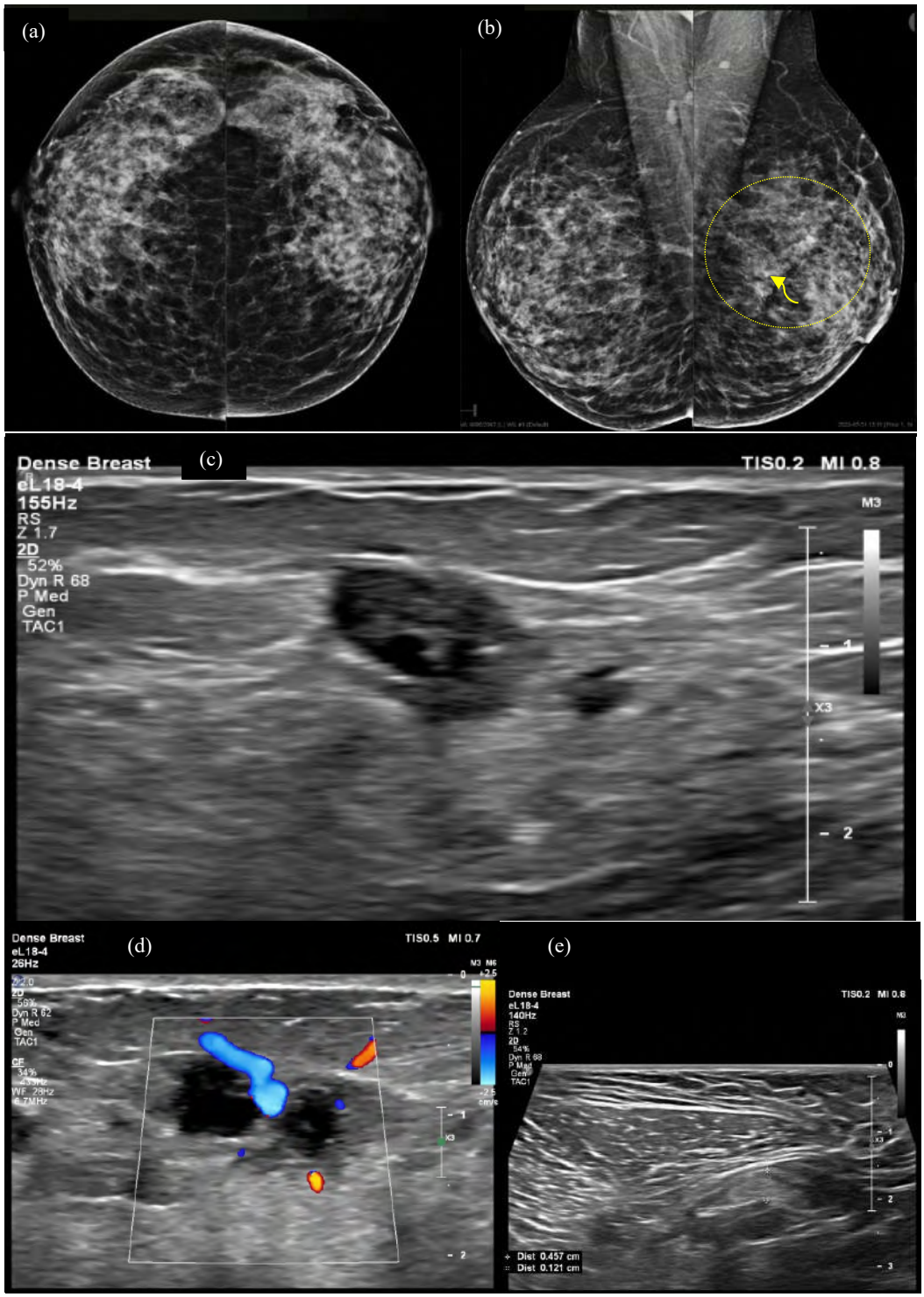


Figure36- (a) Bilateral CC view and (b) MLO view of bilateral breasts (type B) and (c) magnified view of the lesion in MLO view shows three oval-shaped isodense lesions

with partially micro lobulated and partially obscured margins are noted adjacent to each other in the outer central quadrant with multiple punctate and round calcifications (yellow arrow) within the lesions and mild surrounding architectural distortion. (d) and (e) ultrasound images of the left breast revealed a focal area of a cluster of dilated ducts with internal heterogeneously hypoechoic mass, filling it with internal vascularity on color doppler imaging. One dilated duct showing anechoic lumen is also seen extending till the nipple. (f) represents a normal subcentimetric lymph node with maintained fatty hilum and normal cortical thickness.

Case 5: A 39-year-old female came with c/o left breast lump x 2 months. Histopathology & IHC revealed Luminal A molecular subtype with the following imaging findings:



Figures37: (a) Bilateral CC view and (b) MLO view of bilateral breasts (type C) shows no evidence of any mass lesion/suspicious calcification. A focal area of focal asymmetry is noted in upper outer quadrant of left breast with few fine pleomorphic calcifications (yellow arrows). (c) and (d) ultrasound images of the left breast revealed

an irregularly shaped thick, walled complex solid-cystic lesion with micro-lobulated and angulated margins noted in the left breast at 3-4 o'clock position at the skin. The solid component of the lesion appears iso to hyperechoic (occupying approx. 50-60% of the lesion) with multiple tiny internal echogenic foci and mild internal vascularity on CDFI**.** (f) represents a normal subcentimetric lymph node with maintained fatty hilum and normal cortical thickness.

Case 5: A 47-year-old female came with c/o right breast lump with pain and redness x 1 month. Histopathology & IHC of both the breast lesions revealed Luminal B molecular subtype with the following imaging findings:

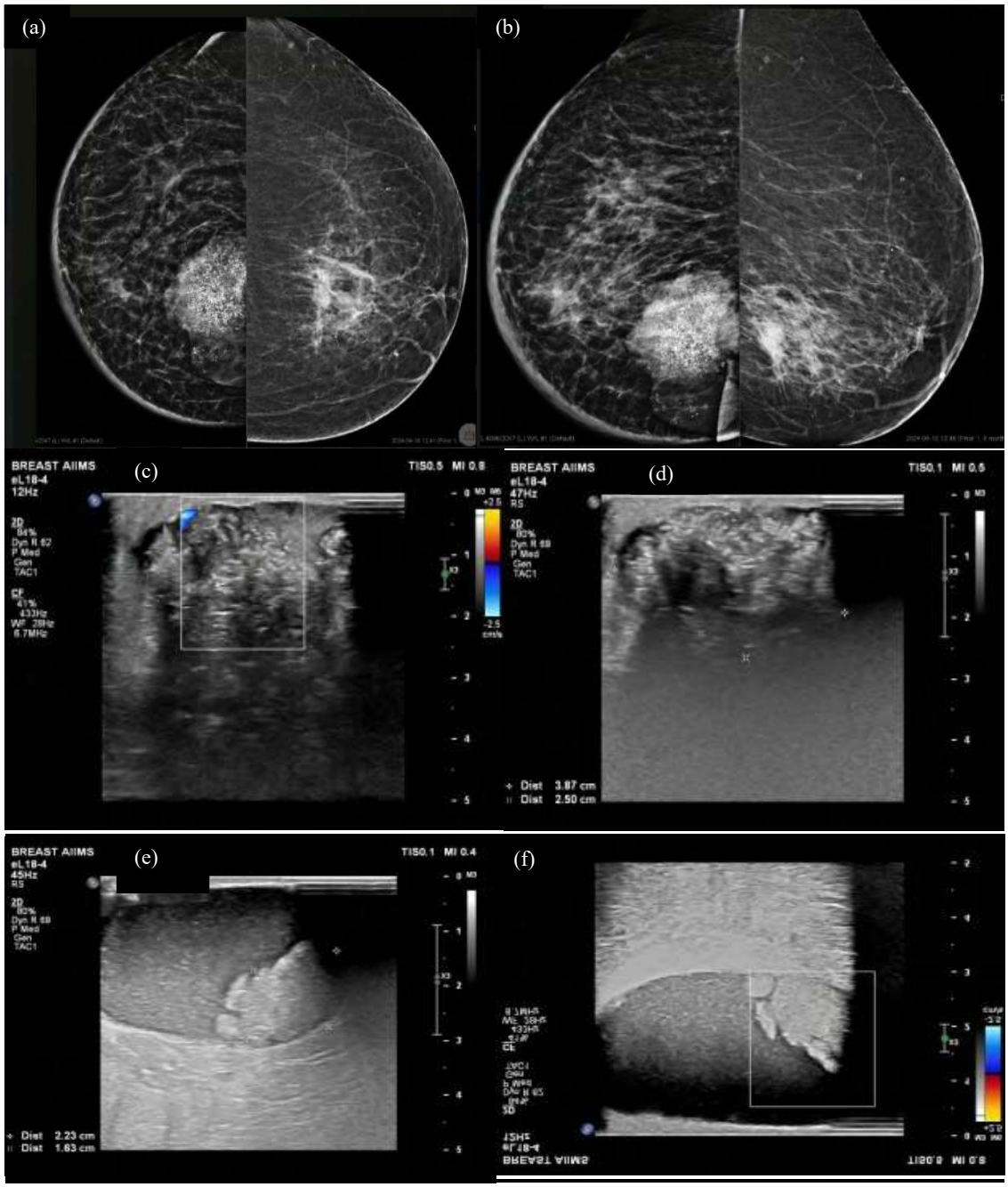


Figure38- (a) Bilateral CC view and (b) MLO view of bilateral breasts (type B)- The right breast shows an irregularly shaped heterogenous lesion with multiple coarse heterogenous and few fine pleomorphic calcifications in the lower inner quadrant. The left breast shows an area of focal asymmetry with few fine pleomorphic calcifications within. (c) and (d), ultrasound images of the right breast revealed an irregularly shaped heterogeneously hypoechoic lesion with internal calcific foci and vascularity with

micro-lobulated margins noted in the left breast at 5-6 o'clock position. Images (e) and (f) show that the left breast shows a well-circumscribed oval-shaped solid cystic lesion with an eccentric solid component of the lesion that appears iso to hyperechoic with mild internal vascularity on color doppler imaging.

Case 6: A 64-year-old female came with c/o Left breast lump x 2 months. histopathological/IHC revealed TNBC molecular subtype with the following imaging findings:

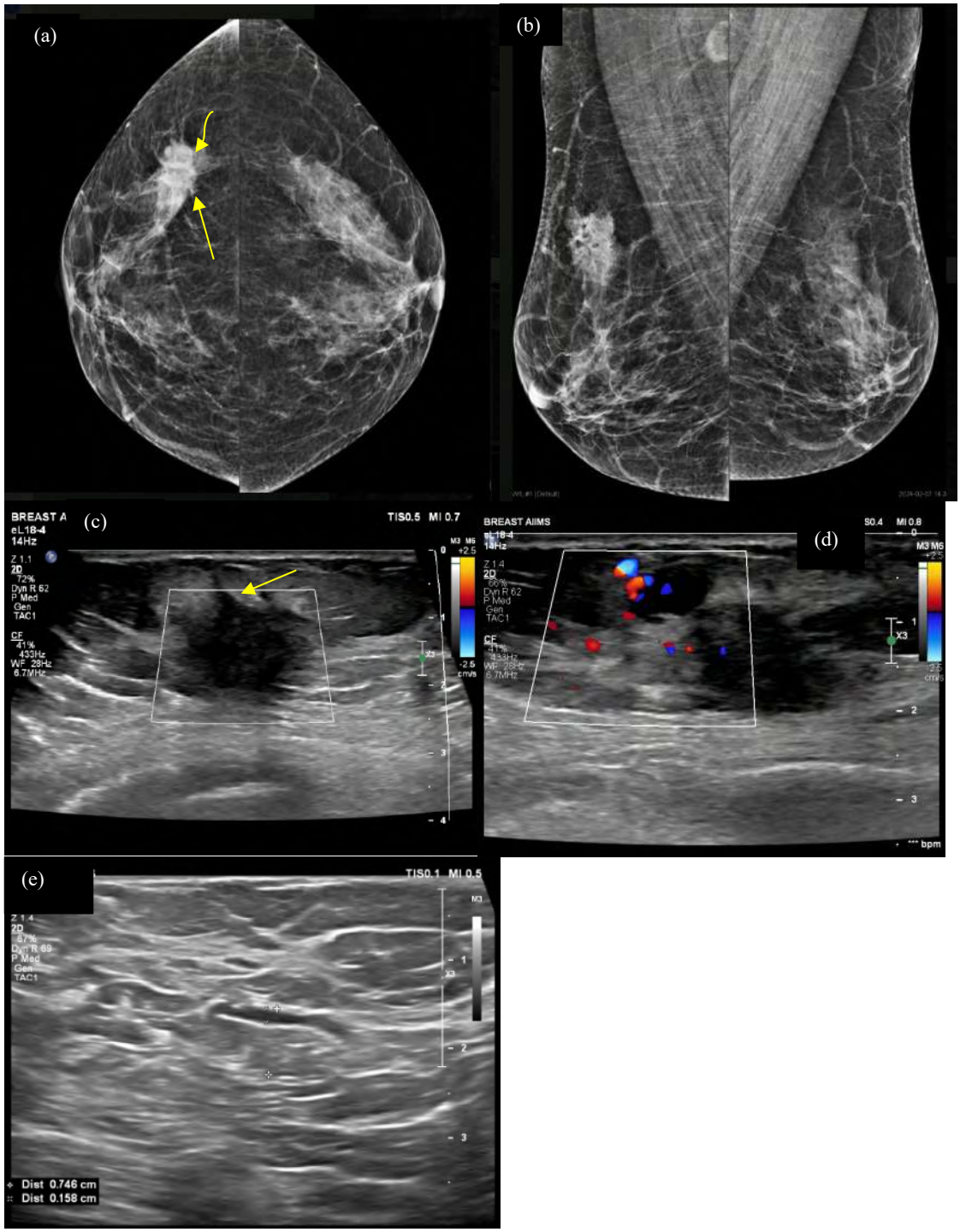


Figure39- (a) Bilateral CC view and (b) MLO view of bilateral breasts (type C)- Right breast shows an irregular shaped heterogenous lesion with partly spiculated (yellow arrow) and partly obscured margins (curved yellow arrow) in upper outer quadrant. No

suspicious microcalcifications noted. (c) and (d), show ultrasound images of right breast which revealed an irregular shaped hypoechoic mass with vascularity on color doppler imaging with spiculations and angular margins is noted in left breast at 11 o’ clock position. Images (e) shows normal subcentimetric lymph node with maintained fatty hilum and normal cortical thickness.

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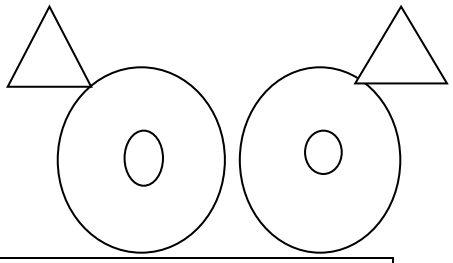
ANNEXURES

Annexures include:

1. Data collection form
2. Participant Information Sheet
3. Participant Informed Consent form
4. Master Chart

1. PATIENT DEMOGRAPHIC AND CLINICAL CASE SHEET

Name:	Age/Sex:	UHID:
Address:	Urban/ Rural	Contact :
Clinical History:	Menarche	
	Menopause	
	Married/Unmarried	
	Age at first child birth	
	Breastfeeding	
	Radiation exposure	
	Family h/o Breast cancer/ovarian cancer/any other cancer	
	Fibroid uterus	
	Cholelethiasis	
	Fatty liver	
	Any other significant history	
Clinical Examination:	Palpable mass Yes/ No	If yes:
	Side:	
	Size:	
	Quadrant:	
	Nipple deviation	Yes/ No
	Nipple retraction	Yes/ No
	Adherence to underlying	Yes/ No

	structures	
	Adherence to overlying structures (tethering)	
	Macroscopic skin changes/ulceration/ satellite nodules/peau ‘d orange appearance	Yes/ No
	Axillary Lymph Node status	
		• Present/Absent • Ipsilateral/ contralateral • Single/multiple
	cTNM Staging	

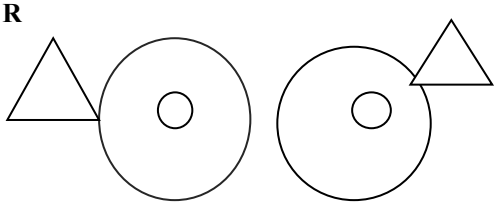
HISTOPATHOLOGICAL FINDINGS	
Histological Grade	
ER	Positive/Negative
PR	Positive/Negative
HER2neu	Positive/Negative
Ki67	High/Low
Molecular Subtype	• Luminal A • Luminal B • HER2neu enriched • TNBC

Luminal A:ER/PR +, HER2neu -, Ki67 <20%
Luminal B:ER/PR +, HER2neu +/-, Ki67 >20%
HER2neu enriched: ER-, PR-, HER2neu +
TNBC: ER-, PR-, HER2neu -

Nottingham modification of Bloom-Richardson system, based on:

(a) tumor tubule formation
(b) number of mitotic figures in most active areas an
(c) nuclear pleomorphism

RADIOLOGICAL DATA:

MAMMOGRAPHY		
Breast composition	**a)** . The breasts are almost entirely fatty **b)** There are scattered areas of fibroglandular density **c)** The breasts are heterogeneously dense, whichmay obscure small masses d) The breasts are extremely dense, which lowers the sensitivity of mammography	
Mass	**SIZE (APxTSx CC diameter)**	
	SHAPE	**1.** Oval **2.** Round **3.** Irregular
	MARGIN	**1.** Circumscribed **2.** Obscured **3.** Microlobulated **4.** Indistinct **5.** Spiculated
	LOCATION OF LESION **R** 	**1.** Laterality **2.** Quadrant and clock face **3.** Depth 4. Distance from the nipple
	DENSITY	**1.** High density **2.** Equal density **3.** Low density **4.** Fat-containing
	CALCIFICATIONS	**Benign or Suspicious** Suspicious morphology • Amorphous • Coarse heterogenous • Fine pleomorphic • Fine linear or Fine linear branching **Distribution of calcifications** • Diffuse • Regional • Grouped • Linear • Segmental
	ARCHITECTURAL DISTORTION	Present/Not present' Location

	ASYMMETRY	• Global asymmetry • Focal asymmetry • Developing asymmetry
	ASSOCIATEDFEATURES	**1.** Skin retraction **2.** Nipple retraction **3.** Skin thickening **4.** Trabecular thickening
AXILLARY ADENOPATHY	1) Present/Absent a. If present then, 2) Single/Multiple 3) Ipsilateral/ contralateral 4) Cortical thickness 5) Hilum 6) Extra Nodal Extension	
NON MASS ASSOCIATED	**CALCIFICATIONS**	**Suspicious morphology** • Amorphous • Coarse heterogenous • Fine pleomorphic • Fine linear or Fine linear branching **Distribution of calcifications** • Diffuse • Regional • Grouped • Linear • Segmental
	ARCHITECTURAL DISTORTION	
	ASYMMETRY	

ULTRASOUND			
Tissue composition	1. Homogeneous background echotexture – fat 2. Homogeneous background echotexture – fibroglandular 3. Heterogeneous background echotexture		
MASS ASSOCIATED	**Shape**	1. Oval 2. Round 3. Irregular	
	Orientation	1. Parallel 2. Not parallel	
	Margin	1. Circumscribed 2. Not circumscribed	• Indistinct • Angular • Microlobulated • Spiculated
	Echo pattern	1. Anechoic	

		2. Hyperechoic 3. Complex cystic and solid 4. Hypoechoic 5. Isoechoic 6. Heterogeneous	
	Posterior features	1. No posterior features 2. Enhancement 3. Shadowing 4. Combined pattern	
	Calcifications	1. Calcifications in a mass 2. Calcifications outside of a mass 3. Intraductal calcifications	
	Associatedfeatures	1. Architectural distortion 2. Duct changes 3. Skin changes 4. Edema 5. Vascularity 6. Elasticity	• Skin thickening • Skin retraction • Absent • Internal vascularity • Vessels in rim • Soft • Intermediate • Hard
	Special Findings		

PATIENT INFORMED CONSENT FORM

प्रतिभागी सूचना सहमति प्रपत्र
[PARTICIPANT INFORMED CONSENT FORM (PICF)]

इस अध्ययन के लिए प्रतिभागी का पहचान नंबरः .....

Participant Identification Number for this Study: .....

.....

Title of Project : **Correlation of Imaging features of Infiltrating Breast Carcinoma on Mammography and Ultrasound with the molecular subtypes (Luminal A, Luminal B, HER2neu enriched and TNBC) and to compare their performance in predicting molecular subtypes.**

शोधकर्ता छात्र का नाम . डॉ ईशुदीप कौर. .फोन नं:.) 9877471118
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शोध मार्गदर्शक का नाम : डॉ। राधा सरावगी गुप्ता
Name of Thesis Guide : Dr. Radha Sarawagi Gupta

मैंने दिनांक ..... के सूचना पत्रक संस्करण II में दिए गए सभी तथ्यों को सावधानीपूर्वक पढ़ लिया है/ मुझे अपनी भाषा में स्पष्ट रूप से विस्तारपूर्वक समझा दिया गया है, और मैंने तथ्यों को पूर्णतः समझ लिया है। मैं पुष्टि करता/ करती हूँ, कि मुझे सवाल पूछने के अवसर प्रदान किए गए। The contents of the information sheet version II dated ,..... that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

मुझे इस अध्ययन की प्रकृति एवं उद्देश्य और इसके संभावित जोखिमों / लाभों तथा अध्ययन की अनुमानित अवधि एवं अन्य प्रासंगिक जानकारी के बारे में विस्तारपूर्वक समझा दिया गया है। मुझे पता है कि इस अध्ययन में मेरी भागीदारी स्वैच्छिक है तथा मैं इस अध्ययन से किसी भी समय बिना कोई कारण बताए, अपना नाम वापस लेने के लिए स्वतंत्र हूं और नाम वापस लेने पर मेरी चिकित्सा देखभाल या कानूनी अधिकारों पर कोई प्रभाव नहीं पड़ेगा। The nature and purpose of the study and its potential risks/benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

मैं समझता/समझती हूं कि इस अनुसंधान में मेरी भागीदारी से मेरे बारे में एकत्र की गई जानकारी और मेरे चिकित्सीय दस्तावेजों के किसी भी भाग को एम्स के जिम्मेदार व्यक्तियों द्वारा ही देखा जाएगा। मैं इन व्यक्तियों को मेरे रिकॉर्ड प्राप्त करने की अनुमति प्रदान करता/ करती हूँ। I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from AIIMS. I give permission for this individuals to have access to my records.

यदि प्रतिभागी बच्चा है तो उसकी मौखिक या लिखित सहमति प्राप्त की गई है। If participants is child, his/her verbal of written assent has been obtained.

मैं उक्त अध्ययन में भाग लेने के लिए सहमत हूं/I agree to take part in the above study.

_____

(प्रतिभागी के हस्ताक्षर / बांए अंगूठे का निशान) (Signature/Left Thumb Impression)

दिनांक/Date……स्थान/Place ……

प्रतिभागी का नाम/Name of the participant……

पिता/पति/पत्नी का नाम /Son/Daughter/Spouse of……

डाक का पूरा पता /Complete Postal Address ……

अगर अशिक्षित हों / If illiterate.

सूचना पत्र को प्रतिभागी को पढ़ा और समझाया गया है और प्रश्न पूछने का अवसर दिया गया है। मैं पुष्टि करता हूं कि व्यक्ति ने स्वतंत्र रूप से अपनी सहमति दी है ।
The information sheet has been read and explained to the participant and given the opportunity to ask questions. I confirm that the individual has given consent freely.

कानूनी रूप से स्वीकार्य प्रतिनिधि या निष्पक्ष गवाह के हस्ताक्षर
Signature of Legally acceptable representative or impartial witness.

दिनांक/Date

एलएआर या निष्पक्ष गवाह का नाम / Name of LAR or impartial witness .

पता / Address :-

प्रमाणित किया जाता है कि उक्त सहमति मेरी उपस्थिति में प्राप्त की गई ।
This is to certify that the above consent has been obtained in my presence.

_____

छात्र शोधकर्ता के हस्ताक्षर दिनांक……स्थान……
Signature of the student Investigator Date …… Place
……

कृपया ध्यान दें: सहमति प्रपत्र तीन प्रतियों में (1) रोगी के लिए, (2) शोधकर्ता, (3) संस्थान के लिए तैयार करें ।

(छात्रों से अनुरोध है कि आसानी से समझ आने योग्य हिंदी अनुवाद स्वयं तैयार करें)

NB: Three copies should me made, for (1) Patient, (2) Researcher, (3) Institution (Students are requested to prepare the translation in simple understandable Hindi on their own.)

PATIENT INFORMATION SHEET

Participant Information Sheet

STUDY TITLE: Correlation between Imaging features of Infiltrating Ductal Carcinoma on Mammography and Ultrasound modalities with molecular subtypes (Luminal A, Luminal B, HER2neu enriched and TNBC)

STUDY INVESTIGATORS: Student: : Dr Ishudeep Kaur, Department of Radiodiagnosis and Imaging, All India Institute of Medical Sciences, AIIMS Bhopal

1. **Guide**: Dr. Radha Sarawagi Gupta, Additional Professor,Department of Radiodiagnosis and Imaging, AIIMS Bhopal

2. **Co- Guide/s :**

   - Dr. Rajesh Malik, Professor and HOD, Department of Radiodiagnosis and Imaging, AIIMS Bhopal
   - Dr. Abhinav Chander Bhagat, Assistant Professor, Department of Radiodiagnosis and Imaging AIIMS Bhopal
   - Dr. Swagata Brahmachari, Associate Professor, Department of General Surgery, AIIMS Bhopal
   - Dr. Deepti Joshi, Additional Professor, Department of Pathology and Lab Medicine, AIIMS Bhopal

3. **Introduction**:

   We are conducting a research study to establish relationship between Imaging features of Infiltrating Ductal Carcinoma on Mammography and Ultrasound modalities with molecular subtypes (Luminal A, Luminal B, HER2neu enriched and TNBC) in AIIMS Bhopal. We would like you to give your full voluntary consent to take part in the study. If you agree to take part voluntarily, we will ask you to sign a consent form.

4. **Purpose of the study:**

The aim of our study is to find a correlation between imaging features of infiltrating ductal carcinoma with its molecular sub-types. This will help us predict the hormonal status of the tumor while its imaging itself and it can fast-track patient management in case of aggressive sub-types like TNBC.

5. Taking part – what it involves:

a. Do I have to take part?

Participation in this research is entirely voluntary. A decision to not take part, or to withdraw at any time, will not affect your rights or medical care in any way.

b. What is expected of me if I take part?

If you participate, we will do your breast cancer imaging and studies as prescribed by your physician already and if required refer you to other department for tissue diagnosis and further management.

How long will my part in the study last?

Your participation in the study would last for single time

Do I have to come for extra visits or undergo extra investigations for the study?

No

c. What are the possible benefits in taking part?

Taking part will help gain knowledge about breast cancer which may result in improvement of management / outcomes for you / patients like yourself.

d. What are the possible disadvantages and risks of taking part?

There are no major foreseeable disadvantages or risks because of the study. There may be risk of loss of privacy and confidentiality of information collected even though all possible measures will be taken to ensure safe data storage and access.

What happens to the information collected from me?

All information collected will be kept strictly confidential and not shared with anyone. Results from the study will be reported as group data and will not identify you in any way.

e. What if I change my mind regarding participation in the study?

You are entitled to change your mind and withdraw at any time during the study without any disadvantage or penalty.

f. Whom do I contact for more information or if I have further questions or concerns?

If you want any information or have questions or concerns, you can contact:

Dr.Ishudeep Kaur
Post graduate student,
Department of Radiodiagnosis and Imaging, AIIMS, Bhopal
Mobile number:9877471118, ishuaiims2016@gmail.com

Dr. Shashank Purwar
Member Secretary,
Institute Ethics Committee – Student Research
(7773010099,
iheepgr@aiimsbhopal.edu.in)

MASTER CHART